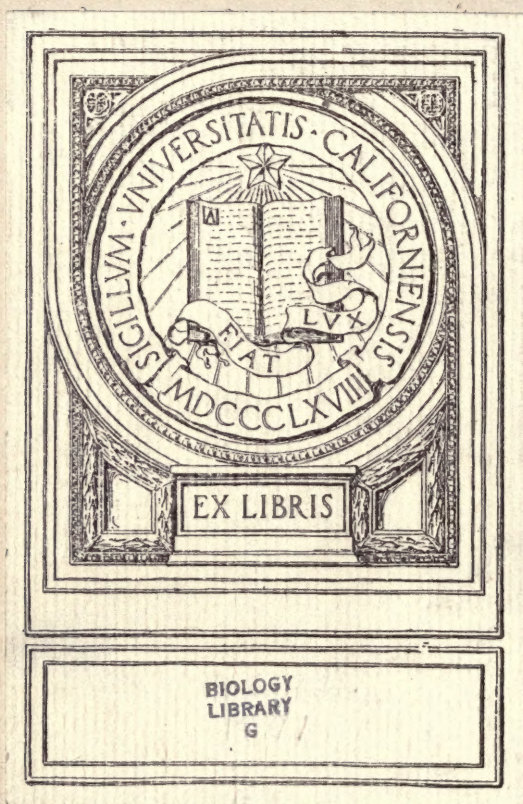


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A COURSE IN NORMAL HISTOLOGY

A GUIDE FOR
PRACTICAL INSTRUCTION IN HISTOLOGY
AND MICROSCOPIC ANATOMY

BY
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PART II.



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SPECIAL PART



PLATE 1

Fig. 1.—Liver-Cell of the Salamander

Fig. 2.—Liver-Cell of the Axolotl

Fig. 3.—Isolated Cells from the Epidermis of the Axolotl

The pages quoted in the text showing "II" in front refer to the pages of Part II. The plain figures refer to Part I.

1. THE CELL

Fig. 1.—Liver-Cell of the Salamander

1000. $\frac{3}{4}$. Sublimate, 3%. Frozen section. *Biondi* solution.

*Liver-Cell of the
Salamander Section.*

If we desire to obtain a typical, clear picture of the structure of the animal cell, it will be most profitable to select the tissues of the tailed amphibia, which distinguish themselves by especially large cells. Among the amphibia the fire salamander, *salamandra maculata*, will give the best results, provided the animals are in a state of good nutrition. Specimens caught early in spring are the best. When bought from a dealer, they should be kept for a few days in a terrarium, well fitted out with damp moss, and should be fed with earth worms. Of the different organs the liver is to be recommended as most efficient.

The animal is killed by severing the head, and the spinal cord is destroyed with a thin wire, taking care not to have one's eyes too near the animal, since the milky secretion, which is spurted at some distance from the cuticular glands, will cause a serious conjunctivitis. After opening the abdominal cavity, the liver is excised and cut into moderately sized pieces, which are placed in a vessel containing 100 cm³ of 3% solution of corrosive sublimate (p. 31). The pieces are now washed overnight in running water and then brought to 5% formalin. After a day they may be cut in the freezing microtome into sections of 10–25 μ in thickness, which can either be stained in *Biondi* solution (p. 67) at once or may first be placed in a fixing soda solution overnight, to remove sublimate coagula. After that they are thoroughly washed in water and finally stained. Mounting is done in Canada balsam or levulose.

A place in the middle of a section is selected, and at once, with even a low power, we will recognize the general structure of the organ, consisting of single cells, arranged in branching arms, similar to spokes of a wheel. Each cell (Fig. 1) has a red stained body, enclosing within it a nucleus of a greenish-blue color. After studying the general aspect of the specimen with low or medium power, we select a favorable field for detail study with the immersion lens.

The cells are polyhedral, corners and edges being rounded off. This can be especially well recognized in thick sections, while thinner cuts will naturally give pure section pictures, viz., squares, pentagons or hexagons with unequally long sides and rounded corners.

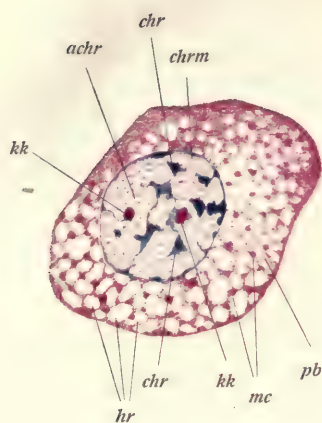


Fig. 1.

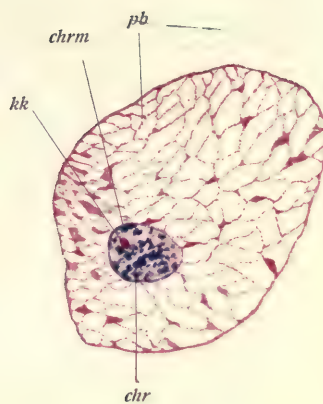


Fig. 2.



Fig. 3.



The Cell-Body.

Studying a single cell-body we notice at once a meshwork design. The entire cell-body consists of red stained meshwork, enclosing small round, respectively spherical spaces. This framework is not evenly constructed throughout the cell-body, but presents essential differences. In some places the bars are more developed than in others, rendering the spaces correspondingly smaller; they may even attain such a size as to obliterate the spaces entirely in certain regions. We generally find that a certain part of the cell-body, near the periphery, is especially dense, the framework gradually becoming looser as we leave this point.

The Protoplasm.

This framework has received the name **protoplasm**. Thus the protoplasmic bars (*pb*) enclose the empty spaces (*hr*) appearing in our specimen; the nature of the latter will be discussed later. They are usually called **vacuoles**, and a protoplasm is spoken of as vacuolized; they are, however, not true vacuoles, i.e., flaws, absolutely separated from their neighbors, but here we have communicating spaces, similar to those of a bath-sponge.

Ectoplasm.

Toward the centre the bars become broader and end at the nucleus, while at the cell periphery they unite to form a layer, which shuts off the cell from the exterior. Such a layer, which must not be mistaken for a membrane such as we have met in the study of the plant-cell, is designated as **ectoplasm** or **crusta**.

The Microsomes of the Protoplasm.

Closer observation of the protoplasmic bars will reveal that they are not homogeneous, but composed of very fine granules, **microsomes**. These are so minute in part that they cannot be discerned, but partly are larger, especially at the point of junction of the bars (*mc*).

The Nucleus,

which will now be discussed, occupies a goodly part of the cell in this specimen—approximately a third or even half. It is spherical in shape and distinctly separated from the cell-body.

Nuclear Chromatin.

The most prominent part of the nucleus is the **chromatin** (*chr*). While the protoplasm has selected one of the acid dyes of the *Biondi* solution, the acid fuchsin, the chromatin has taken the basic methyl green, contrasting in its blue color from all the other elements of the cell. The blue chromatin is found in the interior of the nucleus, in the form of irregular, jagged lumps; furthermore it forms, at the nuclear periphery, a layer, not quite continuous, the so-called **chromatic nuclear membrane** (*chrn*). Connecting the latter with the chromatin lumps are seen **chromatin threads**, also serving to link the various lumps together. The nuclear chromatin thus forms a spherical enclosure bounding the interior of the nucleus, which is,

however, not complete, but perforated in many places. On its inner surface this cell has many excrescences and processes, from which thin threads emanate, interlacing in their course to form a network. At the points of intersection smaller or larger lumps of chromatin are deposited.

The Nucleoli.

Of the remaining elements of the nucleus the nucleoli (*kk*) are the most prominent. They are two small spherical or ovoid bodies in the interior of the nucleus, staining intensely red. They are not found entirely free, but are resting on or, as in our specimen, close to the chromatin-framework. The substance composing them, paranuclein or pyrenin, takes, as we see, the same acid dye as the protoplasm, thereby differing sharply from chromatin.

The Achromatic Substance of the Nucleus.

The remaining part of the nucleus appears filled with a faintly red granular mass, to which the name **achromatic substance** (*achr*) has been given in contradistinction to chromatin and the nucleoli. A closer study of the nature of this substance cannot be made on our specimen, but we may state here that it takes part in walling off the nucleus in the form of the achromatic membrane and is to be found in the interior of the nucleus as fine granules and threads, which in part are intimately mixed with the chromatin.

Fig. 2.—Liver-Cell of the Axolotl¹

1000. $\frac{3}{4}$. Sublimate, 3%. Frozen section. *Biondi* solution.

Liver-Cell of the Axolotl in Section.

As a second object for the study of cell construction we have selected the liver-cell of the axolotl. This animal, easily obtainable from any dealer, is the larva of a Mexican salamandrid, living in water. It can be raised in any aquarium with a good plant supply, propagates even in the larval state, furnishing excellent material for histologic as well as embryological studies.

The care and handling of the animal is the same as that described previously.

After a general examination with low and medium power we again select a part from the middle of the section to be studied with the oil immersion lens.

Fundamentally this picture (Fig. 2) is identical with the first, yet it differs widely, although the liver-cell of the axolotl is larger than that of the salamander. We are the more surprised to find its nucleus to be comparatively small, scarcely measuring one-third the size of the salamander nucleus. We thus learn from this specimen, that a larger cell need not necessarily

¹ *Amblystoma mexicanum*=larva of a Mexican salamandrid.

have a larger nucleus than a smaller cell, i.e., there is no constant relation between the size of the nucleus and that of the cell, neither in closely related animals nor even in the same animal, as we will see later.

The framework of the protoplasm in this specimen is extremely minute, and the interspaces appear relatively large. Here, too, we find a difference in density in different parts of the protoplasm. Microsomes are quite distinct in various places. At the crossing-points in the framework we find aggregations of microsomes, which often give the impression of compact bodies. Again we find the interspaces communicating among themselves.

While the large nucleus of the salamander liver-cell was only sparingly provided with chromatin, we find the small nucleus of the axolotl liver-cell abounding in chromatin; this may go on to such an extent that the entire nucleus is so densely packed, that the achromatic substance is hard to discern, and even the nucleoli (*kk*), which are always present, can hardly be recognized. The chromatic membrane (*chrm*) in such cases is thicker and almost uninterrupted.

Fig. 3.—Isolated Cells from the Epidermis of the Axolotl

600. Isolation by means of acetic acid vapors. *Biondi* solution.

Isolated Cells from the Epidermis of the Axolotl.

Besides the previous sections it is advisable to study an isolation specimen, which in more than one way will complement and further the observations made on the former. Fresh isolated cells can be easily procured by scraping lightly over a freshly cut surface of any organ, taken from a recently killed animal; the tissue pulp collecting on the scalpel is distributed on the slide under addition of a few drops of saline solution.

Since we want to examine stained specimens, we must proceed as follows. A piece of the tail of a living axolotl is snapped off with scissors. This brief operation will not damage the animal, and in a few weeks or months the tail will be regenerated. The specimen is placed on a glass plate, the latter reversed and placed in a properly sized, low glass tray, the floor of which is just covered with acetic acid. After five to ten minutes the vapors of the acid have fixed and loosened the superficial layers of skin, which may easily be completely detached with a scalpel. The small shreds so obtained are put in a watch-glass, and a few drops of *Biondi* solution added (p. 67); after one-half to one hour of staining we transfer to 0.5% solution of acetic acid, where the excess dye is extracted, and now a small particle is mounted in a drop of levulose. Stirring with a needle will isolate the cells easily and completely.

The cells obtained in this manner are of different sizes, but always considerably smaller than the liver-cells. They vary greatly in shape, which can be detected by carefully moving the cover-glass. Partly the cells will adhere together, being conglomerated to larger or smaller masses.

Nuclei show different forms and shapes, and the proportion between the size of nucleus and cell will be inconstant. In the protoplasm we fail to recognize the framework, all appearing granular. Some cells, not all, we notice surrounded by a narrow, lighter layer. Upon closer inspection we notice that this outer layer consists of processes of the cell-body, which serve to connect neighboring cells. These **cell-bridges**, so-called, will receive further consideration.

The nuclei are poorly supplied with chromatin, and the chromatic membrane is very thin.

Leydig's Cells.

Besides the ordinary epithelial cells, we are apt to meet singular, much larger cells, commonly called **Leydig's cells** (*Lz*). The most striking and interesting feature regarding these cells is a well-developed meshwork of fibres, taking a deep red stain. By focussing with the micrometer-screw we convince ourselves that this fibrous structure is situated in the outermost cell-periphery only, enveloping the cell, as it were, with a mantle. The fact is that we are dealing with fibres of protoplasm, which have differentiated themselves in this peculiar fashion in the peripheral layer of the cell-body. In the interior of the cell we find a moderately large, generally irregular nucleus surrounded by a zone of dense protoplasm. The remainder of the cell appears faintly red and structureless. When cells are examined in the fresh state, or after an appropriate vital staining process, this part of the cell will be found to contain a granular secretion. We may regard *Leydig's* cells as monocellular glands, such as are abundantly found in the invertebrates. Among the vertebrates we find them only in the lowest classes, and then only in a state of retrogressive change.

Another important property of the animal cell is illustrated in this specimen. Searching through those places where the cells are not entirely isolated, but cling together in small groups, we will frequently notice between the polyhedral epithelial cells nucleated cells, containing a small amount of protoplasm and possessing most peculiar shapes.

Migrating Cells.

In Fig. 3 the cell (*wz*) is club-shaped. These cells are **migrating**, or **wander cells**; they move actively about, passing between the epithelial cells, and their bizarre shapes are due to the act of squeezing through the narrow intercellular spaces. Most of the cell is occupied by the nucleus, the cell-body being limited to a narrow red band. The morphological changes experienced by such cells must needs principally affect the nucleus, which goes to prove that the nucleus is not a rigid structure, but may adapt itself to surrounding conditions. It is probable that the nucleus itself is capable of executing active movements.

PLATE 2

**Fig. 4.—Migrating Cell from the Lymphatic Peripheral Layer of
the Salamander Liver**

Fig. 5.—Migrating Cells from the Liver of the Axolotl

Fig. 6.—Giant Cells from the Bone Marrow of the Rabbit

Fig. 4.—Migrating Cell from the Lymphatic Peripheral Layer of the Salamander Liver

1000. Sublimate, 3%. Frozen section. *Biondi* solution.

Migrating Cells of the Salamander Liver.

Within recent times a new cellular element has been discovered, in addition to nucleus and cell-body, the **central bodies** or **centriola**, also known as **polar bodies**. They have been demonstrated in many, not all, cells, and are quite favorably represented in our specimens of the salamander and axolotl livers.

Looking over our salamander preparation with low power, we will notice structural peculiarities in the outer zone, i.e., the surface of the liver, different to those which we have found in its interior. Within a more or less extensive zone the characteristic liver structure is lost, the cells failing to arrange themselves in bars, but being irregularly massed together. The cells are considerably smaller. This diminution in size affects mainly the cell-body, which latter also lacks the typical meshy structure of the liver-cell. Let us examine this so-called lymphatic layer with high power.

The nucleus shows no material changes, excepting in shape, which will be more closely considered in the next specimen. The cell-body, as previously stated, consists only of a narrow court around the nucleus, excepting in one spot, where it becomes more extensive and shows a delicate network of protoplasm. A fixed order of arrangement seems to prevail, consisting in concentric fibres, radiating from one point. This centre is situated more or less closely to the nuclear surface, and is represented by a small, intensely red body, which has received the name **central body** or **centriole**. Quite often two centrioles are found in place of one. They lie in close approximation and not infrequently are connected by a thin bridge—the **centrosome**. The central bodies are surrounded by a protoplasmic court, which stains more intensely than the remaining cell-body and is separated from the latter by a thin layer of granules. The entire globular arrangement around the centrioles has been called the **centrosome**. Emanating from it we see the protoplasmic rays approaching the periphery, some following a straight course, others describing a curve around the nucleus. These rays cannot always be distinguished from the rest of the protoplasm, but in favorable cases their more intense staining will render it possible to trace them for some distance.

Archoplasm.

The system of threads described, together with its point of exit, the centrosome, has been called the **archoplasm**, in contradistinction to the remaining protoplasm.

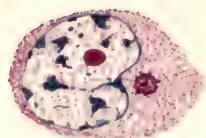


Fig. 4.

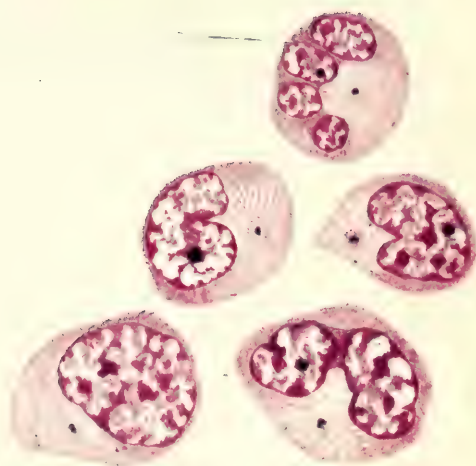


Fig. 5.

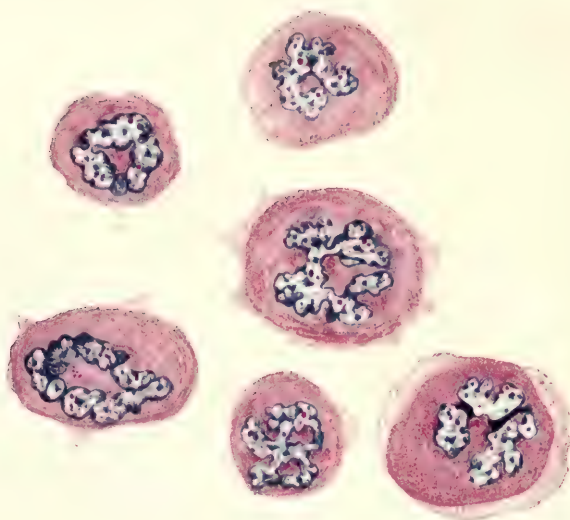


Fig. 6.

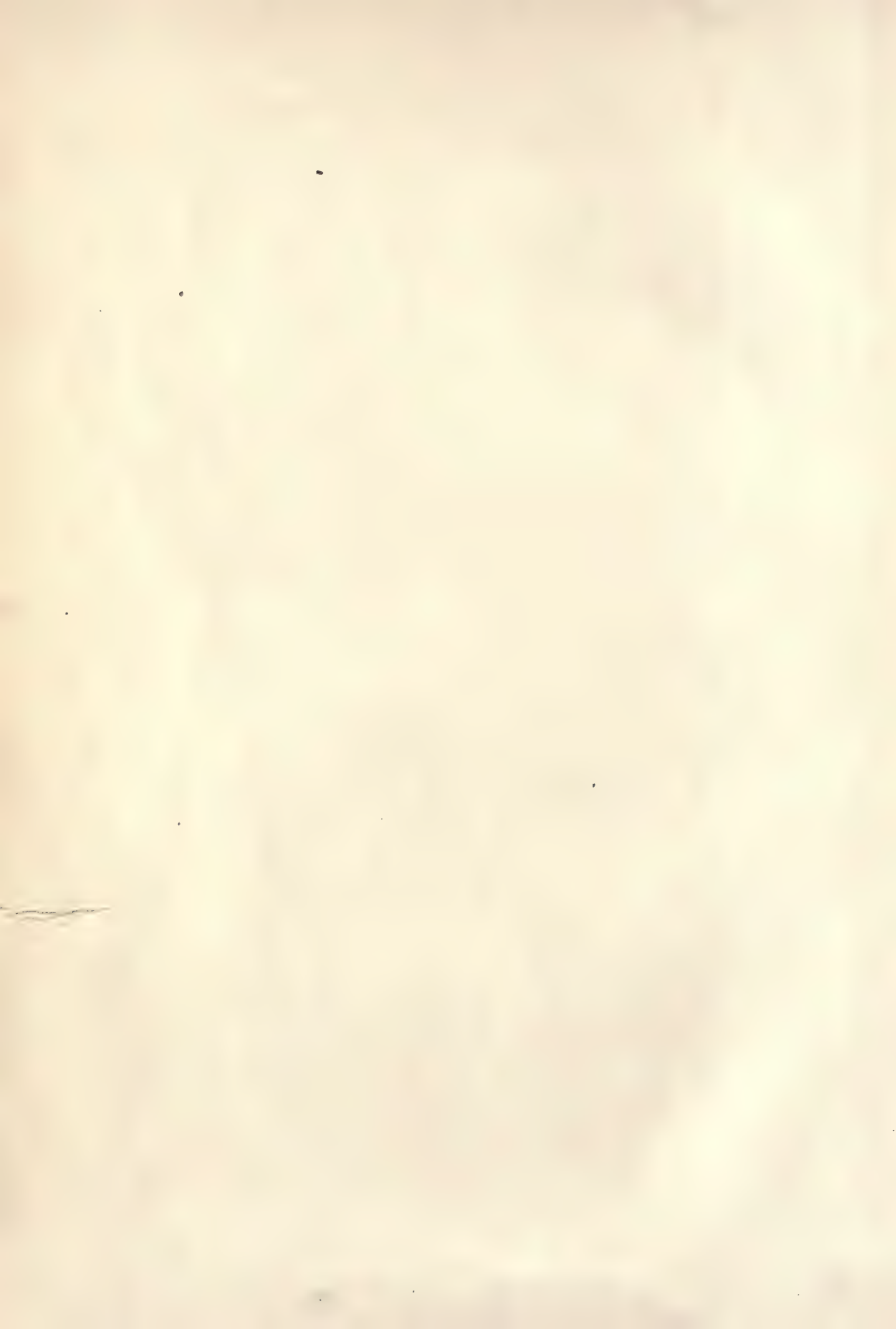


Fig. 5.—Migrating Cells from the Liver of the Axolotl

1000. Sublimate, 3%. Frozen section. Bordeaux R. Iron-alum-hæmatoxylin.

Migrating Cell of the Axolotl Liver.

The migrating cell of the axolotl liver likewise furnishes excellent material for the study of centrioles. The preparation of the slide is the same as before, excepting that we stain the frozen sections differently. They are first stained for from two to three hours with a thin watery solution of Bordeaux R. (p. 65), washed well in water and treated according to the iron-alum-hæmatoxylin method (p. 57). While being reduced in the iron-alum the totally black sections will gradually resume their previous red coloring. We control under the microscope and keep on reducing until the nuclear chromatin has again assumed a pure red color, and the nucleoli only appear black with low or medium power. The sections are washed in repeatedly changed water, dehydrated in the usual manner and mounted in Canada balsam.

We find here, too, a lymphatic peripheral layer under the liver surface, which, if the animal is well nourished, is even more pronounced here than in the salamander. Furthermore, we find the migrating cells abundantly in the blood capillaries between the bars formed by the liver-cells.

When viewed through the immersion lens we recognize at once the deep black centrioles standing out prominently from the pink cell-body. The extraordinarily distinct staining always reveals two of these centrioles, one of which is usually slightly larger than the other. Both are in close approximation, and emanating from them we see the red archoplasm rays radiating to all sides through the cell-body.

Our specimen, while showing centrioles in each of the cells, fails to always bring out the centrosomes. At times we are utterly unable to see any zone around the centrioles; at other times we can detect a more or less distinct court, either colorless or red; again we may find a typical centrosome with its peripheral granular layer. This fact illustrates that centrioles are essential cellular elements in certain cells, while centrosomes form less essential factors.

The Shape of the Nucleus.

Our specimen is furthermore well adapted to the study of the shapes of nuclei, which vary more here than, perhaps, anywhere else. The greater number is round or oval, showing in one place a more or less pronounced indentation. Close to this indentation or in its immediate proximity we usually find the centrioles; the deeper the dent, the more they will be pushed into it. If the formation of this indentation progresses, the nucleus will become bologna-shaped, showing at the same time constrictions, which may go so far as to break up the nucleus into several parts, which are only connected by thin

bridges. In the extremest cases the nucleus will form a ring, having many constrictions, imparting the appearance of a rosary. The centrioles in such cases are situated in the centre.

Fig. 6.—Giant Cells from the Bone Marrow of the Rabbit

800. Sublimate, 3%. Paraffin section. *Biondi* solution.

Giant Cells in Bone Marrow.

Interesting studies in the morphology of nuclei and cells can be made on the bone marrow of young animals. A rabbit, three to four months old, is killed, and one of the legs disarticulated at the hip; the femur is freed from all muscles, sawed at the upper third and fastened in a vise at about the middle. The marrow will exude from the sawed surface; a piece of it is taken on a scalpel and placed in 50 cm³ of 3% sublimate solution for from four to five hours. Treat as usual, embedding in paraffin. Stain with *Biondi* solution (p. 67).

A study of the section with low power will reveal red cells, which excel all the other elements in size. If such a giant-cell is observed under the immersion lens, we find it to have a rounded or oval body, the protoplasm will take a bright red stain, and often we can recognize concentric fibrils. From the cell-body, pointed or ragged processes are seen to emanate, which differ from the cell-body by their pale color.

The nucleus shows most peculiar forms. In one cell it will be in the form of a ring, shaped like a rosary, in other cells the nucleus will be fenestrated; in still others it will have numerous processes. If we combine all these various pictures into one entirety, the resulting average shape will be a half-sphere or basket-form. They may be compared to the fenestrated shells as found among some of the sweet-water heliozoa.

The cell-body is divided by the nucleus into two parts, an intranuclear and an extranuclear. If the former is closely studied, we will find within it, generally in the indentation of the nucleus, numerous small centrioles. They are aggregated into groups, and more than one hundred such central bodies have been counted in one cell.

PLATE 3

Fig. 7.—Liver-Cell of the Axolotl

Fig. 8.—Salivary Glands from the Parotid of the Monkey

Fig. 9.—Liver-Cell of the Frog

Fig. 10.—Pigment Cell from the Skin of the Pike

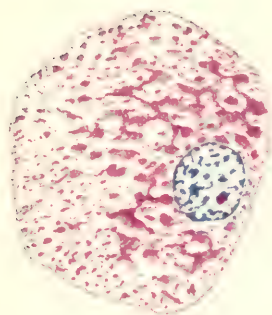


Fig. 7.

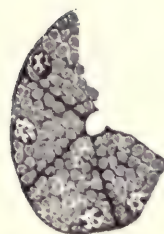


Fig. 8.

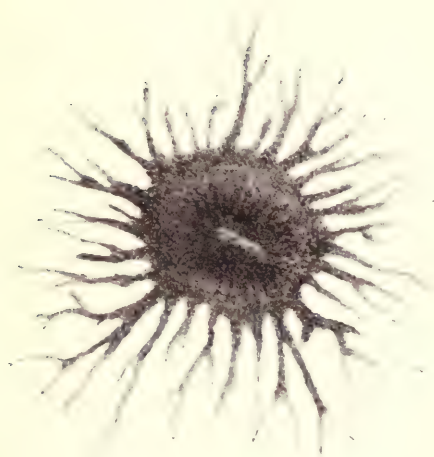


Fig. 10.

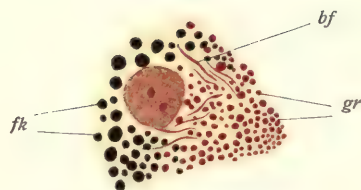


Fig. 9.

Fig. 7.—Liver-Cell of the Axolotl

1000. $\frac{3}{4}$. Sublimate, 5% ; acetic acid, 1%. Frozen section.

Biondi solution.

Liver-Cell of the Axolotl.

The first specimens of salamander and axolotl liver showed the framework or network of the protoplasm. The spaces between it seemed empty, since the treatment given to the specimens rendered it impossible to preserve the substance contained in them. This can, however, be accomplished in the following manner to some extent. Instead of 3% sublimate solution we use 5%, adding 1% of acetic acid. The remaining manipulations are the same as used before.

Since this preservation takes place more completely in the peripheral layers than in the centre, a section closely under the lymphatic peripheral layer is chosen. Again we will find the fine network of protoplasm, but its recognition is decidedly harder, due to the appearance of red-stained granules, lumps and strings, filling out the mesh-spaces more or less completely. We have here, besides the protoplasm, a second ingredient of the cell-body, to which the name **paraplasm** or **deutoplasm** has been given.

Deutoplasm.

Such deutoplasmic element is found in every cell and represents a morphological expression of cellular metabolism. We may regard it as an element denoting either the beginning or the end of cell-metabolism, and, the latter being different in different cells, deutoplasms will vary in their chemical composition.

Glycogen.

Another substance found in liver-cells is the glycogen, which also occurs in many other cells and is formed from the sugar, which the afferent vessels convey to these cells. By placing an unstained frozen section on a slide and adding a drop of Iodin-iodin-potassium (*Gram's*) solution (p. 59) we learn an important microchemical reaction of this glycogen. While protoplasm and nucleus will take a yellow stain, the glycogen will appear deep mahogany brown.

Fig. 8.—Salivary Glands from the Parotid of the Monkey

600. Sublimate, 5% ; acetic acid, 1%. Frozen section. Iron-alum-hæmatoxylin.

Cells of the Salivary Gland.

For further study of the deutoplasm in the animal cell we select the parotid gland of any animal (cat, dog, monkey) or of man. An organ is dis-

sected out and, while yet warm, small pieces are cut out and fixed in sublimate acetic acid. Very thin frozen sections are made and stained in iron-alum-hæmatoxylin (p. 57).

A spot in the periphery of the section is selected, since here the deutoplasmic particles will be best preserved, and an examination is made with the oil-immersion lens. The cells composing the ducts of the salivary gland are small conical bodies, the pointed end looking toward the lumen of the duct. The small round nucleus is situated at the opposite end, the cell-base. The entire cell-body is filled with small globular granules. These are separated by fine separating walls of protoplasm, the latter thus having again the character of framework. These granules likewise are a product of cellular activity; they are formed in the cell and, when required, are expelled and form an important ingredient of the glandular secretion. The details of their origin have not been definitely explained as yet, but it is probable that they originate within the protoplasmic walls and are finally to be traced to the microsomes of the protoplasm.

Fig. 9.—Liver-Cell of the Frog

1000. Osmic acid potassium bichromate. Paraffin section. Acid fuchsin-picric acid.

Liver-Cells of the Frog.

An important deutoplasmic part of the animal cell is found in fat, which we will proceed to study in this specimen. Small pieces of the liver of a freshly caught frog are fixed in osmic acid bichromate solution according to the rules laid down on p. 31 and embedded in paraffin. The sections, which should not exceed 5 μ in thickness, are stained in a color-bath, which is prepared by dissolving 5 gms. of acid fuchsin in 25 cm³ of aniline water (p. 49). A few drops of this solution are placed on the sections, and the slides are heated over a small gas-flame, until the staining fluid commences to steam, when it is allowed to cool. The excess stain is now decanted, and the adhering stain around the section wiped off with a moist cloth. For reduction we cover the sections with a mixture consisting of one part of a 5% solution of picric acid in 95% alcohol, and five parts of 20% alcohol, allowing it to act until the originally bright red sections turn more and more yellow. After thorough rinsing with absolute alcohol we transfer to xylol and mount in Canada balsam.

Fat.

Under high power the conical liver-cells will offer a striking picture. The broad basal part of the cell is filled with black, the apex with bright red granules. In the middle portion both will be found mixed. The black granules are of different sizes and may attain considerable dimensions, while the red are decidedly smaller. The black color is due to the osmic acid used in the fixing process, which has been taken up and reduced by these granules.

This property is mainly peculiar to fat; thus we find in the blackening by osmic acid an important microchemical reaction for fat. In a similar manner, in which many animal cells possess the faculty of storing up or spontaneously manufacturing glycogen, many can take up fat or probably produce it from other carbohydrates; hence we will often meet such fat-granules or globules in the cell-body. The fat found here in the liver-cells is without doubt nutritive reserve fat coming from the bowel, which is conveyed to the cells by the blood-current.

The red granules represent specific products of metabolism in the liver-cell and probably take part in the formation of glycogen.

Altmann's Granules.

According to *Altmann's* theory these red granules represent the living element of the cell; they possess the faculty of synthetically forming fat from complex products and thereby finally turn to fat-globules themselves. According to this hypothesis, therefore, the black granules are an evolution from the red.

Besides the granules we find in our liver-cells thready formations, winding, tortuous, deep-red threads, frequently grouped together in bundles in the vicinity of the nucleus.

Basal Filaments.

Such formations, frequently encountered in glandular cells, are called *basal filaments*. About their origin and function nothing definite can be said as yet.

Fig. 10.—Pigment Cell from the Skin of the Pike

250. Formalin. Frozen section.

Pigment Cells.

In concluding our consideration of the cell-body it will behoove us to mention pigment on account of its wide distribution. For this study we select the skin of a fish. A recently amputated head of a pike is placed in 10% formalin for twenty-four hours; flat pieces of skin are removed with a razor and placed for several hours in 5% formalin. Frozen sections are made and mounted in levulose.

Upon examination we will find in many places, more or less thickly distributed, immensely large cells with numerous processes. The latter arise with a broad base from the cell-body, branch and end in fine points.

The entire cell-body appears light or dark brown in color, excepting a narrow streak in the cell-centre. High power will prove this brown color to be due to numerous granules, which will intensify or lessen the color according to their density. Thus the dye is deposited in granular form in the cell-body. We will see later that this is not always the case, as, for example, it may be present in the form of needles.

The Pigment Circulation.

The main characteristic of the pigment cells is their irregular form and the presence of processes. Responding to certain irritations, the pigment is able to retract from these processes and clump together within the cell-body. This faculty explains the change in color, which certain amphibia, reptiles, fishes, and many of the lower animals are capable of producing in their skin subsequent to some psychic, thermic or photic irritation.

PLATES 4 AND 5

Figs. 11-19:—Blastomeres of the Intestinal Worm of the Horse



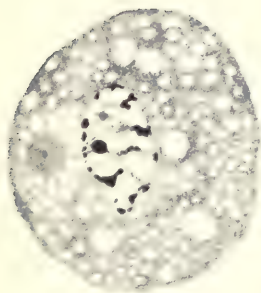


Fig. 11.

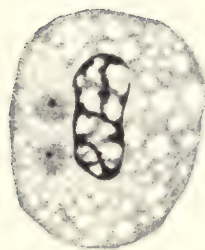


Fig. 12.



Fig. 15.



Fig. 13.

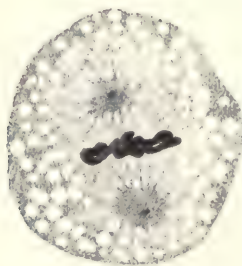


Fig. 14.

Figs. 11-19.—Blastomeres of the Intestinal Worm of the Horse

1000. Alcohol-acetic acid. Paraffin section. Iron-alum-hæmatoxylin.

Indirect Cell Division of Blastomeres of the Horse-Ascaris.

The following specimen will illustrate to us the processes occurring in indirect cell division. Granting that very efficient cell division-specimens may be had from mammalia—and that we will frequently encounter such during our course (one of the most excellent is the amnion of mice—or rat embryos of 2-4 cm length), we prefer to choose an invertebrate, the intestinal worm of the horse, since, on account of the large size of the cells and the small number of chromosomes, the changes take place in almost schematic manner.

The worms may easily be procured from any slaughter-house of horses. They are removed from the bowel of the recently killed animal and transported to the laboratory in a large, wide-mouthed bottle filled with intestinal contents. They can be kept alive in this fashion for two to three days, if kept in a warm place. The female is of sole interest to us. It differs from the male by its greater size and by the form of the posterior end of the body. While the anterior extremity is blunt in both sexes, the tail end of the female tapers. In the male, again, this conical end is twisted to an angle, and frequently small rods, the spicula, are seen emanating from the anal opening, which also serves as sexual cleavage and is situated near the pointed end.

A good sized female is stretched across a basin lined with wax and fastened on both ends with pins. The body-wall is slit open from end to end with slightly opened scissors, spread apart and fixed with pins on either side. We now observe the ovaries, thin thready tubes, arranged in tortuous curves and twists. On disentangling this chaos, preferably with saline, we will come to two thicker uteri, also lengthy tubes, which terminate on their posterior extremity in the ovaries, anteriorly uniting to form a short vagina, which opens on the ventral surface of the animal about the anterior third.

Shortly in front of this opening we transfix the bandlike gut and remove it backward. After cutting around the vaginal opening, including a portion of the ventral wall, we isolate the uteri by careful tension, tie them off with silk at a distance of 10 cm and transfix them posteriorly.

Since the ascaris sheds the eggs shortly after impregnation, we are not apt to find them in the vagina and anterior portion of the uteri, especially not those in the state of segmentation, which are of special interest to us. Previous to fixing the specimen, we wait until the ova have advanced to this state. For this purpose we place it in a vessel, the floor of which is

covered with several layers of well moistened filter-paper. To the edge we apply some vaseline and close the container hermetically with a glass cover. In this moist chamber, at about 15 to 17°, the development will go on, until after about twenty-four hours the eggs will have advanced to the four-cell-state, which is best adapted to our study. Hence the preservation must be postponed to the second day.

Here we must use great care and subtle diligence, if we desire to obtain perfect, unshrunk ova. For fixation we place the specimen in a moderately wide cylinder of proper length, filled with 100–150 cm³ of alcohol-acetic acid (p. 33). The fixation is ended after thirty minutes, the mixture decanted and replaced by absolute alcohol, which is changed after two to three hours. The next day the specimen is cut into several pieces, which are transferred to chloroform, observing the following steps: for three hours they are kept in a mixture of 1 part of chloroform and 4 parts of absolute alcohol; at intervals of three hours we decant one-fourth of the mixture, substituting an equal amount of chloroform. The pieces remain in chloroform in a well-covered, properly sized vessel overnight. The next day the transfer to paraffin should be made with the most scrupulous care. The uncovered vessel is placed in an oven, and every half hour a piece of soft paraffin is added, according to how quickly the chloroform evaporates. The entire evaporation should be completed overnight, and we now place the pieces in a different vessel, filled with fresh soft paraffin, cooled to the melting-point, and place the same in the paraffin oven. After half an hour the soft paraffin is replaced by hard paraffin, and after another half hour the block may be prepared, setting the different pieces closely together in parallel order, so that each cut will contain several cross-sections of the uteri. The staining of the sections, which should be about 10 μ in thickness, is done according to the iron-alum-haematoxylin method (p. 57), with or without primary staining in Bordeaux R. (p. 65). *Biondi* staining will also give excellent results (p. 67).

Under low power the wall of the uterus appears lined with elongated cells, which are club-shaped at their free end. The lumen of the organ is filled with eggs, spherical formations, enclosed in a thick homogeneous shell. In their interior we find, according to their state of development and the plane of section, different numbers of large cells, the **blastomeres**, or segmentation-cells. We will proceed to study cell division on these blastomeres, first examining a certain state with medium power, after which we will observe the same state more detailed under the immersion lens. Considering the large number of ova, we are pretty sure to find all the various states, the primary being the rarest.

Resting Cells.

Fig. 11 shows a blastomere in the resting state. The cell-body is studded with numerous larger or smaller vacuoles. It encloses an irregular nucleus, the chromatin of which forms chromatic membrane, chromatin lumps and threads connecting the latter. Nucleoli are present, mostly single. Closely to the nucleus we see a single centriole, surrounded by its centrosome; radiating from the latter we recognize, though often indistinctly, the archoplasmic rays extending to all sides.

The Prophase.

The changes first experienced by the cell, occurring in the nucleus as well as in the archoplasm, are preparatory to the actual segmentation, and have therefore been designated as the **prophase**. Fig. 12 shows the first state of development. The changes in the nucleus are not very marked, but still perceptible. A consolidation of the chromatin has taken place. Where we had lumps and threads before, we now find the latter thickened to such a degree that they cannot be differentiated from the former. The changes occurring in the archoplasm are still more striking. The formerly single centriole has divided itself to form two. These have separated some distance, each being surrounded by a centrosome, from which a system of rays emanates. The rays of both systems partly cross, partly are lost in the protoplasm of the egg, a number of them will join to form spans between the two centrosomes. These latter rays are called **central spindles**, the former **polar spindles**.

In Fig. 13 changes in the nucleus are most prominent. The chromatic membrane has disappeared, the irregular jagged chromatin-bars of the first state have become more even and smoother, and finally have joined to form a regular long thread, comprising the entire chromatin mass of the former nucleus, winding in a tortuous course within the now open nuclear space. At this point of development, as is sometimes the case in the preceding stage, the nucleolus has disappeared, the centrioles have separated still more and are approaching the poles of the nucleus.

They will reach there during the following stage (Fig. 14). Like two suns, they stand opposite each other, sending their rays in all directions. The thread of chromatin has undergone segmentation, and its components, the **chromosomes**, have arranged themselves like a plate about the equator of the two systems of rays. From this phenomenon this stage has received the name of **equatorial plate**.

The changes occurring in the chromatin thread will be still better illustrated in such cuts, where the section has been made vertically through an axis interposed between the two centrioles, which necessarily would comprise the equatorial plate itself, while the centrioles would be cut off above and below. Here we will find (Fig. 15) that the thread has changed to four hooklike, bent chromosomes, which have arranged themselves in irregular stellate form, the *monaster* or mother-star. The clearness and simplicity of these pictures are due to the small number of chromosomes present, which in another variety of ascarides might sink to half the number, while in the mammalia it rises to twenty-four.

The Metaphase.

The prophase has now been concluded, and the cell enters the actual segmentation stage, the **metaphase**, wherein the most important fact consists in the splitting lengthwise of the four chromosomes. From the four mother-chromosomes eight daughter-chromosomes are formed. The splitting process, even in the ascaris, must take place very rapidly, since it is rarely seen under the microscope. Again pictures, such as Fig. 16 shows, are not infrequently

encountered. Here we find the eight daughter-chromosomes, although not parallel, still closely together.

The separating of the chromosomes is shown in Fig. 17, four chromosomes on either side approaching their respective pole, while synchronously five archoplasmic threads are spread between the corresponding daughter chromosomes, so that we now have three different varieties of threads. **Polar threads**, entering the cell-body from the centrosome, the **mantle fibres**, spread between centrosomes and chromosomes, and the **central spindle fibres** connecting the opposing daughter-chromosomes. Often during the separation of the chromosomes it happens that their free extremities will approach, thus forming a barrel-shaped figure, but soon the bands will rearrange themselves in star-shape and we now have two stellate figures, the *dyaster* or the daughter-stars.

Anaphase.

The cell now enters the **anaphase**, which ushers in the first signs of the **division of the cell-body** (Fig. 18). The latter takes place in a plane, which is vertical to the axis connecting the two central bodies, the division being brought about by a gradual constriction from without inward. During this process the central spindle fibres are compressed to a bundle, which grows constantly smaller. Simultaneously retrogressive changes occur in the daughter-chromosomes, the ends of which melt into one chromatin-thread, which becomes irregular and jagged. A nuclear membrane forms, and finally (Fig. 19) we find two separate, closely approximated cells, each having a nucleus and a centrosome, containing a centriole. Nucleoli reappear during the formation of the chromatin thread.

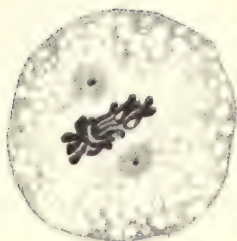


Fig. 16.

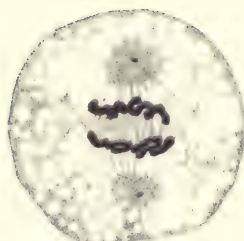


Fig. 17.



Fig. 18.

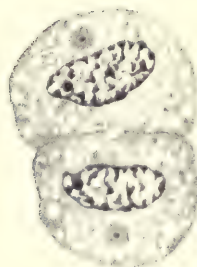


Fig. 19.



PLATE 6

Fig. 20.—Epithelium from the Mesentery of a Newborn Rat

Fig. 21.—Cuboid Epithelium from a Frog's Kidney

II. THE TISSUES OF THE ANIMAL BODY

1. EPITHELIUM

Fig. 20.—Epithelium from the Mesentery of a Newborn Rat

200. Surface specimen. Silver nitrate. Hæmalum.

We will select the simplest form of epithelia to begin with, the flat epithelia, as found in large body cavities and in the entire vascular system. The best material will be found in the abdominal cavity, and specifically in the mesentery or the great omentum. Newly born, or at least very young animals are preferable to old, since in the latter the presence of fat interferes and the epithelial lining is generally incomplete. The best subjects are newly born rats or guinea-pigs. The animal is killed by decapitation, the abdominal cavity opened by a median longitudinal incision, the great omentum is turned upward, a fold of intestine is resected with its mesentery, carefully spread on a wax plate and the gut fixed with hedgehog bristles. The wax plate, with the specimen, is now placed on a flat plate and a 0.75% watery solution of silver nitrate slowly poured over it. After approximately five minutes the plate, with the opaque, milky specimen, is placed in a large vessel containing water, where it is cleansed of excess solution and later is transferred to fresh water. The specimen is now freed from the wax plate under water and is exposed to light in a white plate, preferably to direct sunlight. The latter will reduce the silver salts in from ten to fifteen minutes with the production of a brown color. It will take correspondingly longer in diffuse daylight. After the specimen has attained a good brown color, the water is decanted, and after an additional washing with water hæmalum is substituted (p. 56). After ten minutes, which is sufficient time for the nuclear staining to take place, the excess dye is thoroughly washed out with hydrant water. While still in the latter, the wax plate is again placed under the specimen, which is fastened thereupon and the water replaced by 50% alcohol, followed in a few minutes by 70% and later by 90%. This will render the specimen so hard that it can be detached from the bowel and cut into pieces of appropriate size, which can be dehydrated completely in absolute alcohol and put through xylol to be mounted in Canada balsam.

Flat Epithelium.

A specimen prepared in this manner will present a network of fine dark brown lines, making numerous minute curves, appearing as a fringe. The fact is, that the silver nitrate has only blackened the cement, which binds the cells together, i.e., it has reduced the silver salt, while the cells themselves have taken but a light brown color. The cells are very irregularly shaped,

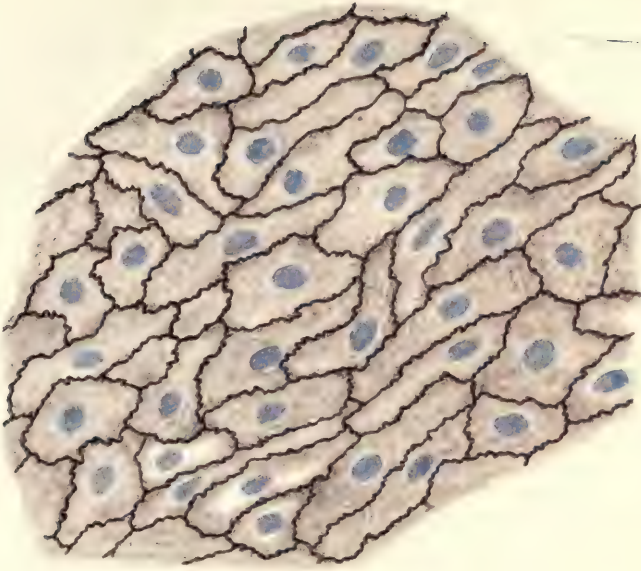


Fig. 20.

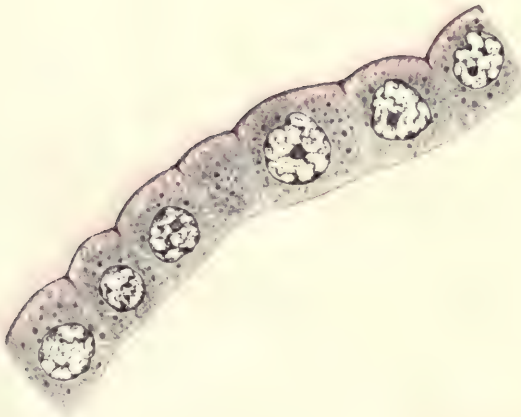


Fig. 21.

thin plates, linked together by toothlike processes. Each cell contains an oval or round nucleus. By searching through different depths of the specimen by the aid of the micrometer-screw we will detect another network-picture, situated deeper than the first and also provided with nuclei. We thus learn that the mesentery contains two layers of epithelium, separated by a minute layer of connective tissue. When reaching the intestine, these layers separate and, enclosing it, form its outer covering.

Fig. 21.—Cuboid Epithelium from a Frog's Kidney

600. Sublimate-nitric acid. Paraffin section. Hæmatoxylin-iron-alum.
Acid fuchsin.

Cuboid epithelia are found in wide distribution in the animal body, especially forming the lining of numerous glands. We will study them on the frog's kidney. We select a female, where we recognize two elongated, dark reddish-brown bodies as the kidneys; after folds of intestine and ovaries have been pushed to the side, they are seen lying closely behind the colon at both sides of the vertebral column, separated only by the large vessels. The excised organ is divided into three to four longitudinal sections, which are fixed in 50 cm³ of sublimate nitric acid for from three to five hours (p. 31). Embedding takes place in paraffin. Sections should be 5 μ thick and be stained in iron-hæmatoxylin (p. 56). After reduction we wash well in running water and counterstain in a 0.1% solution of acid fuchsin (p. 64). The sections are stained until a light red color results and are dehydrated immediately.

Cuboid Epithelia.

The kidney tubules of the frog for the greatest part of their course are lined by simple cuboid epithelium, which, however, is modified in different parts. Let us select a portion of the second part. Here we find cuboid cells, which mostly measure the same height as width. The cells are mounted on a thin, red membrana propria. Each contains a globular, not quite regular nucleus. The cell-body shows an indistinct network of protoplasm with numerous coarse and fine microsomes.

The Cilia.

The free surface of the cell is slightly convex and is lined with a pink band, which under closer observation proves to be made up of minute short rods in juxtaposition. Such formations are frequently found on the free surface of epithelial cells, occurring in different degrees of development. We designate such rods as **cilia**, and call epithelia provided with such cilia **ciliated epithelia**. In other parts of the frog's kidney we find long, motile flagella in place of the short unelastic rods.

The Cement Substance.

We notice in our specimen another peculiarity of epithelial tissue. The cells are, as we see, closely approximated. Their relation may be so intimate

as to render it impossible for us to recognize their dividing line. However, we find that at the free surface there is a sort of wedge inserted between the cells, which takes the hæmatoxylin lake especially well. Plastically considered, these formations must form a system of wedges, connected among themselves and surrounding the head of each cell in net-fashion, thus separating each cell from its adjacent fellows. They have been termed the **cement wedges** or cement substance of epithelium, and will be encountered in most varieties of epithelial tissue.

PLATE 7

Fig. 22.—Pigmented Epithelium from the Eye of the Horse

Fig. 23.—Pigmented Epithelium from the Eye of the Hawk

Fig. 24.—Pigmented Epithelium from the Eye of the Hawk

Fig. 22.—Pigmented Epithelium from the Eye of the Horse

280. Surface specimen. Chromic acid, $\frac{1}{50}\%$.

The following three specimens will present epithelium, which might be classified among the cuboid epithelia, but shows very peculiar modifications of the latter: the pigment epithelium of the eye. In order to first get a good surface view, we use the eye of a larger animal, in the following manner: With a razor all the coats of the eyeball are incised along the equator, i.e., parallel to the periphery of the cornea; with scissors we complete the separation, thus dividing the globe in an anterior and posterior half. The vitreous humor is removed from the latter and the specimen placed in very diluted chromic acid, 1–2 cm³ of a 1% solution of chromic acid to 50 cm³ of water. The following day the retina has become detached from the other layers and is removed by transfixing it at the point of entrance of the optic nerve, the *papilla nervi optici*. In reality we have not removed the entire retina, but only its inner layer, physiologically the most important part. The outer layer will adhere to the middle coat of the eye, the choroid. This pigmented layer we can also remove after two or three days more, by shaking the specimen vigorously, while still in the solution. The epithelium will be found floating in the liquid in the form of larger or smaller pieces. These are transferred by means of a wide pipette into water, which must be changed frequently; one piece is selected and brought to a slide in a like manner and mounted in levulose. The pieces may previously be stained in diluted hæmalum (p. 56).

Pigmented Surface Epithelium.

A very regular picture presents itself. The cells have taken a brown color; they are pentagonal or hexagonal in shape and separated from their fellows by light dividing lines. In the centre of most cells a lighter spot can be observed. With high power we notice that the brown color is due to small oval or rod-shaped brown granules, which are deposited in the cell-body in large quantities. In one part of the cell they are lacking or at least are only thinly spread. At this place we find, as a stained specimen will demonstrate, the nucleus, which is free from pigment. The light lines between the cells represent the **cement substance**, connecting the cells among themselves.

The Brownian Molecular Motion.

After teasing a shread of epithelium in a drop of water with two needles and covering it with a cover-glass, we can observe that this insult to the tissues has torn several cells apart. Some of the pigment granules have be-

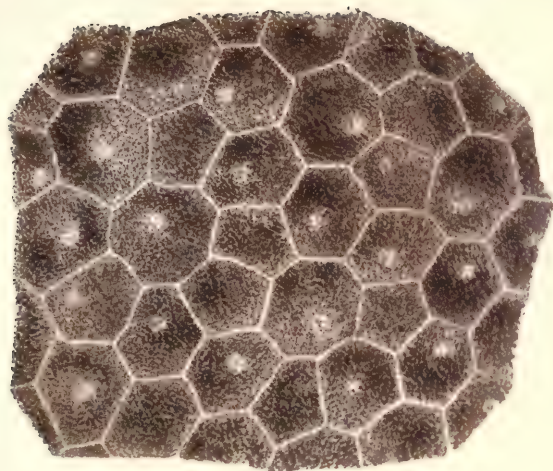


Fig. 22.

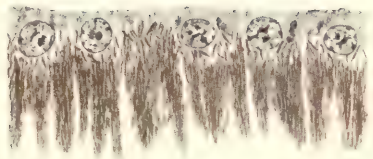


Fig. 23.

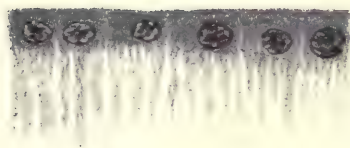


Fig. 24.



come free and are swimming around in the field. Besides floating in the currents, created by evaporation of water at the edges of the cover-glass, the granules will execute another independent motion: they dance to and fro, constantly tumbling and turning upon their own axis. After cooling the slide, by the application of cold water, the motility will be found to be diminished, while careful warming will accelerate the same. This **Brownian molecular motion**, which may also be found in living cells, must be regarded as a purely physical process, a visible expression of the invisible motions constantly executed by the molecules of every liquid.

Fig. 23.—Pigmented Epithelium from the Eye of the Hawk

600. Sublimate-nitric acid. Paraffin section. Iron-alum-hæmatoxylin.

The preceding specimen may give the impression that pigmented epithelia are simple flat cells, similar to those of the peritoneum. This is not so, since we are dealing with cells having processes which project into the elements of the inner layer of the retina. In the mammalia these processes are very fine and tear off very easily. The eye of the bird is better suited for their demonstration. The eye of any bird is enucleated and placed in sublimate-nitric acid (p. 31) for five hours, washed overnight and transferred for three days into 10% formalin. We now dehydrate carefully, and while still in absolute alcohol hood-shaped pieces of the globe are cut with the razor and embedded in paraffin. The sections are made transversely and stained by the iron-alum-hæmatoxylin method (p. 57).

Transverse Section of Pigment Epithelium.

Under low power we search for the pigment epithelium, which is then examined under high power. The cell-body is cuboid, slightly flattened; in contradistinction to that of the horse, it contains little pigment. This is, of course, true of many mammalia also, where the outer zone of the cell-body is free of pigment. The bulk of the pigment is found in the long processes which extend into the interior of the retina. The pigment here occurs in a different form to that found in mammalia; it does not form granules or rods, but is found in the form of needles. A cement substance between the heads of cells cannot be demonstrated in the bird.

Fig. 24.—Pigmented Epithelium from the Eye of the Hawk

600. Sublimate-nitric acid. Paraffin section, bleached. Iron hæmatoxylin.

In order to ascertain whether these processes are really protoplasmic extensions of the cell-body, the pigment must be removed from the cells, i.e., the specimen must be bleached. For this purpose the sections are placed for one hour

in a 0.1% solution of permanganate of potassium, to be followed with a mixture of equal parts of 1% sulphide of potassium and 1% oxalic acid. The specimen, first stained brown, will be decolorized by the latter solution. It is thoroughly washed in hydrant water, and, without cover-glass, the bleaching is controlled under low power. If the bleaching is not entirely satisfactory, the process may be repeated. Having been washed thoroughly the sections should be stained with iron-hæmatoxylin (p. 56).

*Bleached Pigment
Epithelium.*

The cell-body will now stain intensively, and the pointed or jagged processes emanating from it can easily be recognized.

PLATE 8

**Fig. 25, *a* and *b*.—Cylindric Epithelia from the Uterus of a
Monkey**

Fig. 26.—Epithelium from the Intestinal Villi of a Dog

Fig. 25, a and b.—Cylindric Epithelia from the Uterus of a Monkey

100. Sublimate-*Mueller's* fluid-acetic acid. Paraffin sections. Iron-alum-hæmatoxylin.

Our next consideration will be given to simple cylindric epithelia, as found in a high stage of perfection in the lining of the uterus. The uterus of a child or a monkey should be chosen. The organ, taken from a fresh body, or from the chloroformed animal, is cut with a sharp razor into three pieces—fundus, body and cervix—carefully avoiding compression. The pieces are fixed in 150 cm³ of the mixture mentioned on p. 32: sublimate, *Mueller's* fluid and acetic acid, for from five to six hours at a temperature of about 40°. Hereafter they are washed in the usual manner, dehydrated and embedded in paraffin. Cross-sections are made of the body and stained with the iron-alum-hæmatoxylin method (p. 57).

Simple Cylindrical Epithelium.

The epithelium of the endometrium is in form of a single layer of quite regular cylindrical cells. It would be more exact to call them prismatic cells, since most cross-sections will not show a circle, but an irregular pentagon or hexagon. The longitudinal diameter of the cells measures from three to six times the length of the cross-diameter. The cell-body shows an indistinct network of protoplasm, which is densest at the base of the cell, becoming lighter toward the free surface.

The irregular ovoid nucleus is situated near the base, or may advance as far as the centre of the cell. It adapts itself to the shape of the cell, becoming more elongated in thin cells, more rounded in thicker cells.

Centrioles in the Cylinder Cells.

Between the heads, i.e., the free ends of the cells, we find well-developed cement wedges (*kl*). Of much greater interest is the fact that this specimen shows distinct central bodies in the head of the cell (*ck*). They are represented by two minute bodies, situated closely together and surrounded by a small lighter zone. Thus these centrioles show an essentially different position from those in the migrating cells of the salamander liver (Plate II, Fig. 4), where they were always found in the immediate proximity of the nucleus.

Not infrequently we see a cross-section through the surface of the cell. If the section is made right through the head of the cell (Fig. 25, *b*), we will find within each of the polygonal cells our two centrioles, generally sit-

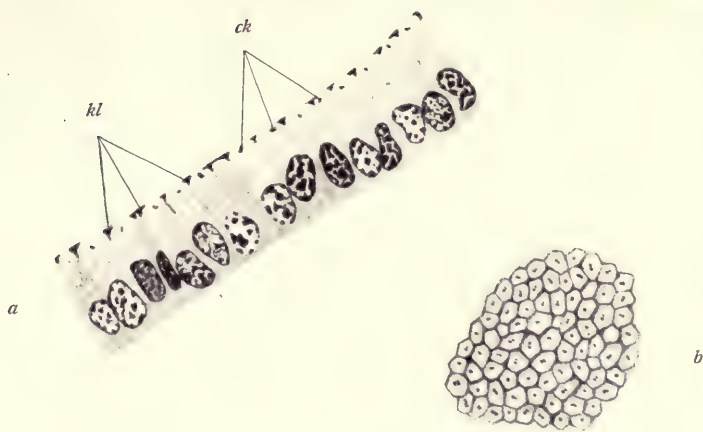


Fig. 25.

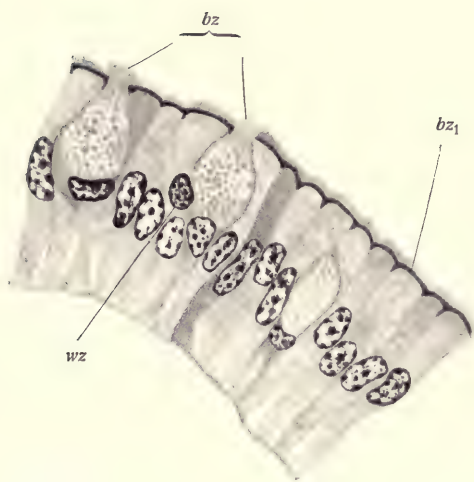


Fig. 26.



uated quite in the centre. Each cell is surrounded by a strong, black line, the cement or sealing frame, and, where several cells come in contact, this cement substance is present in large quantities.

Fig. 26.—Epithelium from the Intestinal Villi of the Dog

600. Sublimate-chromic-osmic-acetic acid. Paraffin section. Iron-alum-hæmatoxylin.

A further representative of cylindrical epithelia is found in the lining of the intestinal tract. We select any recently killed mammal (cat, dog, monkey, preferably one which has starved for one to two days), open the abdomen, resect a piece of the small intestine, place it on a wax plate and open it longitudinally, fastening the piece at once with hedgehog bristles, before the muscles have time to distort the specimen by their contraction. Intestinal epithelium is very sensitive and must therefore be guarded against any laceration; for this reason it is not advisable to rinse before the specimen has been fixed. Fixation takes place by putting the plate, specimen side down, in a vessel containing fixing solution, upon which it floats. For intact preservation of the epithelial tissue we recommend especially combinations of sublimate, osmic acid, chromic acid, and acetic acid, such as the mixture spoken of on page 32. Fixation takes place from twelve to twenty-four hours. Wash, dehydrate, embed in paraffin, and stain with iron-alum-hæmatoxylin (p. 57).

Under low power we notice on the inner surface of the intestine long, fingerlike projections, the villi. The best place for examination with high power will be found near the free end of a villus.

Cylindrical Epithelia, Ciliated.

Each villus is covered by a layer of cylindrical epithelium, made up of long, palisadelike cells. The cells are mounted on a homogeneous membrana propria. They arise by a base, which is slightly broader, but tapers directly. The narrow cell-body again widens out as it approaches the free surface. Here and in the centre of the cell the epithelia are closely approximated, but at the foot of the cells interspaces are often seen.

Goblet Cells.

Between these narrow cells we find more or less frequently large swollen cells. They arise with a broad base from the basement membrane, become constricted for a short distance and again widen out considerably. At the free surface the cell ends in a greatly narrowed mouth. Their shape has earned them the name of **goblet cells** (*bz*).

Besides their shape these cells offer another characteristic peculiarity. The wide part of the cell, the goblet proper, appears crowded with quite large granules, becoming indistinct as they approach the surface and forming a confluent homogeneous mass at the mouth, appearing like a stopper

projecting into the lumen of the gut. Their object is to manufacture the intestinal secretion; they are secretory cells. Their secretion is the mucin, the granules in the interior of the goblet representing a pre-stage of mucin.

While glancing over the epithelial cells we find, aside from the typical cylindrical cells, all sorts of transitional forms, approaching the goblet-shaped cells. First we notice in the superficial third of the cell-body a few granules, which gradually increase in number (bx_1), distending the cell-body by their presence, until finally the cylindric cell has changed to a typical goblet cell.

The Cilia.

On the free surface we find in the intestine likewise a lining of cilia, similar to those found on the cuboid epithelia of the frog's kidney. On the cell-surface we notice a thin, deep black layer, which is continuous with the cement wedges, separating the cell-heads. We may regard this as a **cuticular formation**, from which the cilia arise. The latter are shorter and thicker than those of the kidneys; they are not of uniform length, thus giving the epithelial surface an irregular, jagged appearance. They probably play an important part in the absorption of the nutritious substances passing through the intestine. As soon as production of secretory granules in the epithelial cell has reached a certain degree, i.e., as soon as the cylindric cell has changed to a goblet cell, the contents rupture through the ciliated lining, the secretion thus pouring freely into the lumen of the intestine. Hence the continuity of the cilia will appear broken, wherever fully developed goblet cells are found.

The nuclei are of irregular, oval form, generally situated in the centre of the cell, but often changing their position in either direction. Again the rule holds true, that nuclei will adapt their shape to their surroundings, their long axis generally being at right angles to the long axis of the cell.

Migrating Cells in the Epithelium.

Numerous **migrating** cells are found in the intestinal epithelium. They are cells which have left the blood and lymph vessels and pierced through the epithelial layer to be finally found in the lumen of the gut. Wz , in our figure, represents such a cell. They make their way between the cells, increasing the intercellular spaces and impinging on the calibre of the cell-body, so that they often appear to be resting within the substance of the cell.

PLATE 9

Fig. 27.—Epithelial Cells from the Intestine of a Monkey

Fig. 28.—Intestinal Epithelium of the Pond Mussel

**Fig. 29.—Ciliated Cells from the Trachea and Nares of the
Monkey**



Fig. 27.

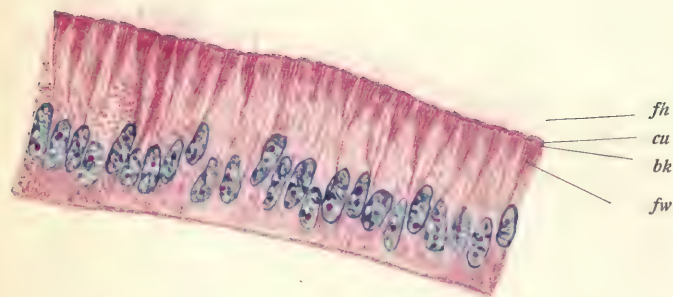


Fig. 28.

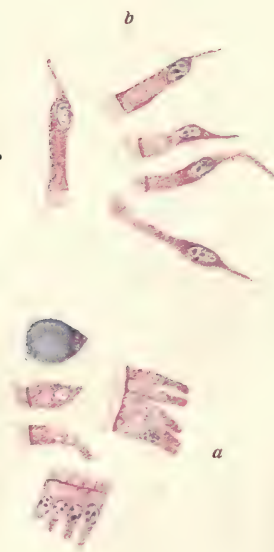


Fig. 29.

Fig. 27.—Epithelial Cells from the Intestine of a Monkey

280. Isolation specimen. Acetic acid vapors. *Biondi* solution.

We will now study the epithelial cells of the intestine in an isolation specimen, selecting the previously (p. II, 7) described method. The piece of gut is spread on a wax plate in the same manner as in the preceding specimen, and is exposed to acetic acid vapors.

The picture will correspond in all essentials to that of the section specimen, excepting that perchance no goblet cells are seen. Partly the cells are entirely isolated, partly they adhere to others in little groups. We have an especially good view of the basal end of the cell in this specimen. We also notice a migrating cell (*wz*), which is just passing through the epithelium.

Fig. 28.—Intestinal Epithelium of the Pond Mussel

600. Sublimate-Mueller's fluid-acetic acid. Paraffin section. *Biondi* solution.

A typical specimen for the study of ciliated epithelium is furnished by the ordinary pond mussel, *Anodonta cygnea*, the gills and intestines of which possess magnificent ciliated epithelia. It is of advantage to anæsthetize the animal before opening its shell, which can be accomplished by placing it overnight in a vessel containing a 1% solution of chloral hydrate. The next day the shell will be found open; the hinge-band is severed and half of the shell removed by dissecting off the closing muscle. After one half of the enveloping coat has been removed and the external gills turned back, the lower part, containing the bulk of the intestinal tract, is freely visible. The latter are delivered, split with the razor, and one-half is fixed in 100 cm³ of sublimate-Mueller's fluid-acetic acid for five hours (p. 32). The usual after-treatment and embedding in paraffin follows. Very thin sections are made and stained with *Biondi* solution (p. 67); the iron-alum-hæmatoxylin method (p. 57) likewise furnishes very clear pictures. The remaining half of the specimen can be prepared for isolation in the manner described on p. II, 7 by means of acetic acid vapors.

Cylindrical Ciliated Epithelia.

The long, narrow cylindrical cells of the intestinal epithelium are mounted on a homogeneous basement membrane. The nucleus is situated near the base of the cell, always containing one or even two very prominent nucleoli.

The free surface of the cells is covered with a narrow, homogeneous cuticular band (*cu*), slightly arching from cell to cell, from which arise the fairly

long, fine, weakly staining cilia (*fh*). Between cuticular layer and cells a single row of fine granules may barely be distinguished, the **basal granules** (*bk*), one being provided for each cilium. It is very interesting to note in this specimen that the cilia are continued into the cell itself, converging, after piercing the cell, to form a tapering train within the cell-body. This so-called **root** of the cilia (*fw*) can be traced throughout the entire length of the cell; it passes close by the nucleus, to become lost at the base of the cell.

Fig. 29.—Ciliated Cells from the Trachea and Nares of the Monkey

280. Isolation specimen. Acetic acid vapors. *Biondi* solution.

The ciliated epithelium, as found in the upper and intermediate respiratory tracts of the mammalia and of man, is not made up of simple cylindrical epithelia, as we will soon be able to verify; our reason for selecting it at this time is solely with regard to the ciliated cells.

The trachea of a freshly killed mammal is exposed and a piece resected, split in the long axis and mounted on a wax plate, the mucous membrane of course being face-up. Isolation and staining is done according to the rules on p. II, 7. To obtain the ciliated epithelium of the nares, the head is sawed in two, close to the median line, after the brain has been removed; the two halves are turned apart and the cartilaginous septum narium can be excised and treated in the same manner.

Isolated Ciliated Cells.

In the specimen of trachea (Fig. 31, *a*) we will see narrow cells, tapering toward their base and surmounted by moderately long, densely distributed cilia with distinct basal granules. The oval nucleus lies close to the cell-base or in its centre.

The ciliated cells of the respiratory mucous membrane (Fig. 31, *b*) are longer and narrower. Their basal ends taper still more. Here, as well as in the trachea, we notice between the ciliated cells, elements which we immediately recognize as goblet cells.

Goblet Cells Among Ciliated Epithelia.

The entire goblet has taken a deep blue stain, the nucleus appears closely adhering to the base. The cell-body is drawn out to a point. Mucin granules cannot be recognized among the deep blue contents of the goblet; our primitive fixing method has failed to preserve them. On the other hand an important staining property of mucus is illustrated well in our specimen, namely, its affinity for basic dyes, which it takes on eagerly.

PLATE 10

**30.—Flagellated Epithelium from the Vestibule of the
Lamprey**

Fig. 31.—Stratified Epithelium from the Human Cornea

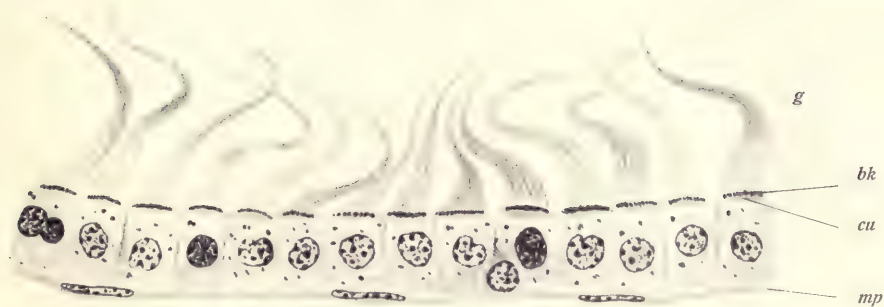


Fig. 30.

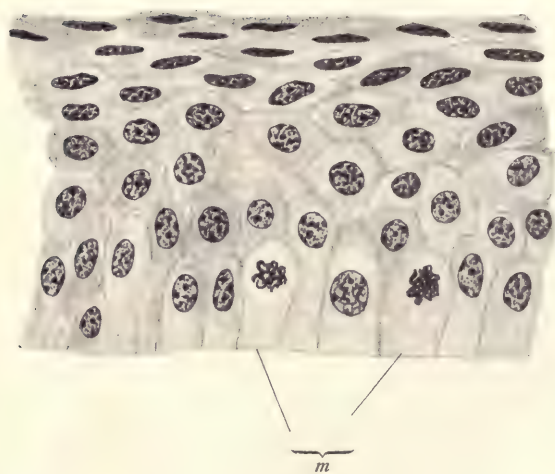


Fig. 31.

Fig. 30.—Flagellated Epithelium from the Vestibule of the Lamprey

600. Alcohol-chloroform-acetic acid. Paraffin section. Iron-alum-hæmatoxylin.

A wonderful object for the study and life observation of ciliated epithelium is furnished by the lamprey, *petromyzon fluviatilis*, in the epithelial lining of the largest part of its aural capsule. The animals can easily be captured in the spring, when they travel up stream, for the purpose of depositing their eggs, and at other times may be secured from dealers. The animal is killed by severing the head about three fingers' breadth behind the eyes. Beginning at the dorsal edge of the eye, we carry a longitudinal incision through muscles backward, which will soon bring us to a pea-sized prominence, the aural capsule. Dissecting away all muscles, we cut the capsule smoothly at its base and fix for fifteen minutes in alcohol-acetic acid or alcohol-chloroform-acetic acid, according to the rules discussed on p. 33. We embed in paraffin and stain the sections with iron-alum-hæmatoxylin (p. 57).

Observation of the Ciliary Motion in the Living Object.

The aural capsule of the other side is used for observation of the ciliary motion *intra vitam*, razor sections being made and examined in normal saline. The excessively long tapering cilia appear like a pointed whip. The ciliary motion is slow, the different whips moving in coordinate order, without separating from one another. The movements are rhythmical, comparable with the waving of a wheat-field before the wind.

The stained specimen should first be regarded through low power. If the section has been made at right angles to the long axis of the capsule, i.e., the entire animal, and traverses the centre of the organ, we find the ciliated epithelium only in a sinus, bulging out laterally and on the ventral surface, the vestibulum so-called, while the remaining portion of the capsule is covered by a different type of epithelium.

Cuboid Flagellated Epithelium.

High power will reveal cuboid to low cylindrical cells, mounted on a *membrana propria* (*mp*) containing granules. Sometimes a cell is seen pushed between two adjacent cells at their bases, its head never reaching the free surface. The cellular protoplasm forms an exquisite network, containing deeply stained granules. The nucleus, often double, is round and occupies the centre of the cell.

On the free surface of each cell we notice a lenticular, pale-staining *cuticula* (*cu*), which near the lumen is studded with a single layer of

fairly thick *basal granules* (*bk*), which may be so densely distributed as to resemble a black band. From each basal granule arises one *cilium*. The latter is thin at the point of origin, increasing in thickness and again tapering at the pointed end. While the cilia arise separately at their base, they coalesce more or less in their course, forming a sort of whip (*g*), which ends in a point. In the same manner, as they are seen moving together in life, the different cilia making one whip are always found closely approximated in the fixed specimen.

Roots, as we have seen them in the anodonta, cannot be distinguished here.

Fig. 31.—Stratified Epithelium from the Human Cornea

600. Formalin. Frozen section. Iron-alum-hæmatoxylin.

Stratified epithelium, next to be considered, is found in wide distribution forming the outer covering of the body of vertebrates, penetrating to some extent into the body-openings. A typical example is the cornea of man or of the larger mammalia. The globe is enucleated *in toto*, fixed in 10% formalin, transferred the next day to 5% formalin, where the cornea is separated with the razor close to the corneo-scleral border and divided into several sections, which are cut transversely on the freezing microtome. Staining with iron-alum-hæmatoxylin (p. 57).

On its outer surface the cornea is bounded by stratified epithelium. The deepest layer consists of cylindrical cells, varying in length and width, which protrude into the next layers by their club-shaped peripheral ends. The nucleus is most always situated in this clublike extremity, so that we see a basal protoplasm stratum void of nuclei. Quite often we find cells in the state of division (*m*).

Stratified Flat or Squamous Epithelium.

Following these cylindrical cells are two to three layers of irregular polyhedral cells, the first layer having long tapering processes which project between the heads of the cylindrical cells. Outside of these there are three layers of flat epithelium, the cells becoming flatter as they near the surface, so that the outermost layer consists of large polygonal plates, which give a smooth contour to the organ.

Such epithelium is designated as stratified flat, sometimes, though not quite correctly, as pavement epithelium. It is found, at times, having many more layers, on the entire body surface, the mouth, pharynx, vagina, fossa navicularis of the male urethra, the entire female urethra and certain parts of the respiratory apparatus.

PLATE 11

**Fig. 32.—Cell-Bridges from the Epithelium of the Human
Glans Penis**

Fig. 33.—Bladder Epithelia of Child

Fig. 34.—Bladder Epithelia of Monkey

**Fig. 35.—Respiratory Epithelia from the Nares of the
Guinea-Pig**

Fig. 32.—Cell-Bridges from the Epithelium of the Human Glans Penis

1000. Alcohol. Paraffin section. Methyl violet. *Gram's* anilin xylol.

In our third specimen (p. II, 8) we have already met with cellular bridges. They are still better developed among the stratified epithelia, especially among the thick and fast-growing epithelia. We select a piece from the surface of the glans penis; or, better still, from some fast-growing cuticular excrescence, e.g., warts or pointed condylomata. The pieces are fixed in absolute alcohol (p. 33) and embedded in paraffin. Very thin sections are made and stained in gentian violet (p. 59) for from fifteen to thirty minutes, the excess dye is rinsed off with water, and a few drops of *Gram's* solution (p. 59) are placed upon the section for a half to one minute. After decanting the latter, the slide is dried carefully around the specimen, and the latter itself dried with tissue paper, using threefold paper with simple pressure and without moving sideways. A mixture of anilin and xylol, equal parts, is placed on the sections, which will extract the stain powerfully; when the parts appear light blue, the reduction is discontinued. After washing well in pure xylol we mount in Canada balsam.

Intercellular Gaps and Bridges.

We notice in our specimen that the cells in the thick epithelial strata, especially in the middle and deepest layers, are separated by moderately large intercellular gaps. From cell to cell fine threads are spread, which are continued into the cell-body.

Epithelial Fibres.

We thus have a system of fibres, running from cell to cell, piercing the peripheral parts of the cells; the fibres may not only connect two adjacent cells, but may cover a long distance, piercing a great number of cells in their course. Midway between two cells we usually find a small knot deposited in each fibre. By close congregation of the knots of the various fibres, lines, appearing like strings of beads, are created, which run through the interstices of the cells parallel to the contour of the latter.

Fig. 33.—Bladder Epithelia of Child

300. Formalin. Frozen section. *Biondi* solution.

Another variety of stratified epithelium is the so-called transitional epithelium. To demonstrate it, we select the bladder. After excising the

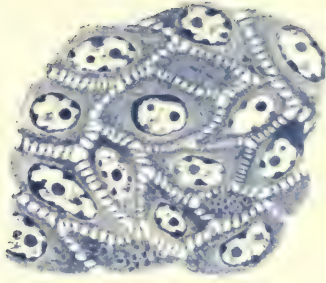


Fig. 32.

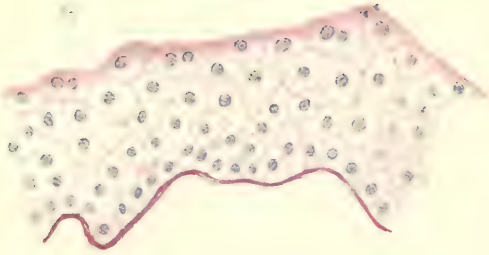


Fig. 33.

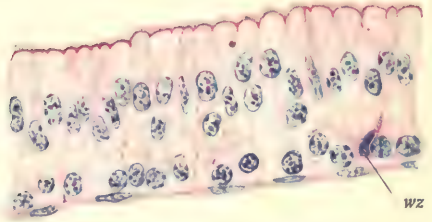


Fig. 35.

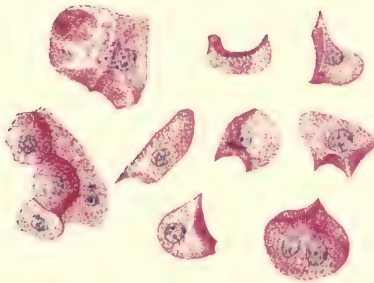


Fig. 34.

viscus, we open it longitudinally; when dealing with smaller mammalia, the entire organ, when larger mammalia or the human specimen are used, pieces of it are mounted on a wax plate, the mucous membrane being uppermost. Fixation in formalin. Staining of the frozen sections in *Biondi* solution (p. 67).

Transitional Epithelia.

We find here a picture which has many similarities in common with the stratified flat epithelia. The number of layers varies, averaging six to eight. The deepest layer consists of cylindrical cells, which, however, are more irregular and less deep than those seen in the stratified squamous epithelium. Following this are four to six strata of entirely irregular, polyhedral cells, which do not become flatter as they approach the free surface. The main characteristic of these cells is seen in the uppermost layer, forming the lining of the bladder lumen. It is made up of large cells, which project into layers below by variously sized processes.

Thickness and Shape of Epithelial Cells Dependent upon the State of Contraction of the Bladder.

The thickness and shape of bladder epithelium is in a high degree dependent upon the state of contraction of the organ. Our specimen shows a state of medium contraction. In a distended bladder our epithelia would appear decidedly flatter and broader. In order to illustrate this physiologically not unimportant phenomenon, we withdraw the urine from a freshly killed, preferably female, animal with a glass catheter and refill the bladder with a 10% formalin solution. For this purpose we attach to the glass tube, after complete catheterization, a rubber tube, into which a funnel is inserted. Raising the glass tube to a level with the funnel, we fill the entire apparatus with the formalin solution, close the rubber tube with a pinchcock, insert the glass catheter into the bladder per urethram and release the stopcock. After one hour the abdomen is opened, the bladder tied off and cut off distant to the ligature. The distended viscus is now suspended in a 5% solution of formalin, where it is left until the following day, when it will be ready for sectioning on the freezing microtome.

Fig. 34.—Bladder Epithelia of Monkey

400. Isolation specimen. Acetic acid vapors. *Biondi* solution.

In the manner described before, the fresh organ is attached to a wax plate and treated according to the method described on p. II, 7.

Isolated Transitional Cells.

The singular form of bladder epithelium is illustrated better in this isolation specimen than in section. The cell-body is seen studded with projections, pointed and jagged, which serve to connect adjacent cells. The knowledge

of the shapes of these cells is of importance in practice, since they are to be looked for in the urine during pathological conditions of the mucous membrane of the bladder.

Fig. 35.—Respiratory Epithelia from the Nares of the Guinea-Pig

500. Sublimate-acetic acid. Frozen section. *Biondi* solution.

The last variety of epithelium to be considered is the stratiform cylindrical, as found in ciliated form in the upper respiratory tract. The head of a small mammal is divided with the saw near the median line. On the larger half we will find the cartilaginous nasal septum, which is excised with scissors and suspended in a mixture of 3% sublimate with 1% acetic acid for five to six hours. Washing must be continued for twenty-four hours in running water, after which the specimen is placed in 5% formalin. Thin frozen sections are made transversely to the surface and stained in *Biondi* solution (p. 67).

Stratiform Cylindrical Ciliated Epithelium.

The most prominent element of this specimen are long ciliated cylindrical cells, such as we have previously seen isolated (Fig. 31, *b*). We notice in our section that their finely drawn-out basal ends reach the membrana propria, and are at that point set in between other conical cells. The heads of the latter do not reach the epithelial surface. These latter cells are usually designated as **replacing cells**, their object being to replace the ciliated cells, which have been used up in their activity and are therefore of limited vitality.

As the ciliated epithelia reach throughout the thickness of the layer, we cannot speak of stratified epithelium, hence we name epithelium, having several rows of nuclei stratiform epithelium.

PLATE 12

Fig. 36.—Subcutaneous Connective Tissue of the White Rat

Fig. 37.—Subcutaneous Connective Tissue from the White Rat

**Fig. 38.—Cross-Section through the Ligamentum Nuchae
of the Calf**

2. CONNECTIVE TISSUE

Fig. 36.—Subcutaneous Connective Tissue of the White Rat

650. Sublimate. Frozen section. *Biondi* solution.

As a type of loose connective tissue we select the subcutaneous connective tissue of a small mammal, preferably that of the rat. The animal is decapitated and the skin over the thorax and abdomen is slit open in the median line and dissected off to the sides, taking in all the loose connective tissue which binds the skin to the muscles. A glass syringe, equipped with a metal needle, is filled with 3% sublimate solution, and the subcutaneous tissue is injected therewith. When pressing down the piston, the subcutaneous tissue will disengage in the form of a bladder; we have thus created an artificial oedema and simultaneously fixed our tissue. A fair-sized piece of hide is cut off, mounted on a wax plate and placed in a 3% solution of sublimate for two to three hours. The specimen is washed overnight and transferred to 5% formalin for twenty-four hours. After removing the skin, frozen sections of moderate size are made. They are stained with *Biondi* solution (p. 67) and mounted in balsam or levulose. It might be worth mentioning that the syringe must be washed and cleansed directly after use.

Our sections will present an exceptionally complete and instructive picture of connective tissue and its component parts.

Connective Tissue Fibres and Fibrils.

Due to the pressure of the injected fluid the *connective tissue fibres* have been greatly separated. They are seen (*colf*) in the form of tortuous thicker or thinner fibres winding their way across the field, being composed of numerous delicate *fibrils*, which at times is much more distinctly shown than in this picture.

Elastic Fibres.

Besides these collagenous fibres we meet numerous thinner fibres, equally tortuous but deeper red than the previous; in these a longitudinal striation cannot be distinguished. They are the **elastic fibres** (*elf*). These two varieties of fibres, aside from their difference in staining properties, as will be shown later, are differently affected by acetic acid. To demonstrate this, we will prepare another specimen, treating it in the same way as the first, except that we use normal saline for injection instead of sublimate. With curved scissors we cut off the roof of the oedematous swelling and place it on a slide under a cover-glass. The collagenous fibres will appear about the same as before, although the cellular elements will be less prominent. We now lift our cover-glass and supply a few drops of 2% acetic acid. At once a swell-

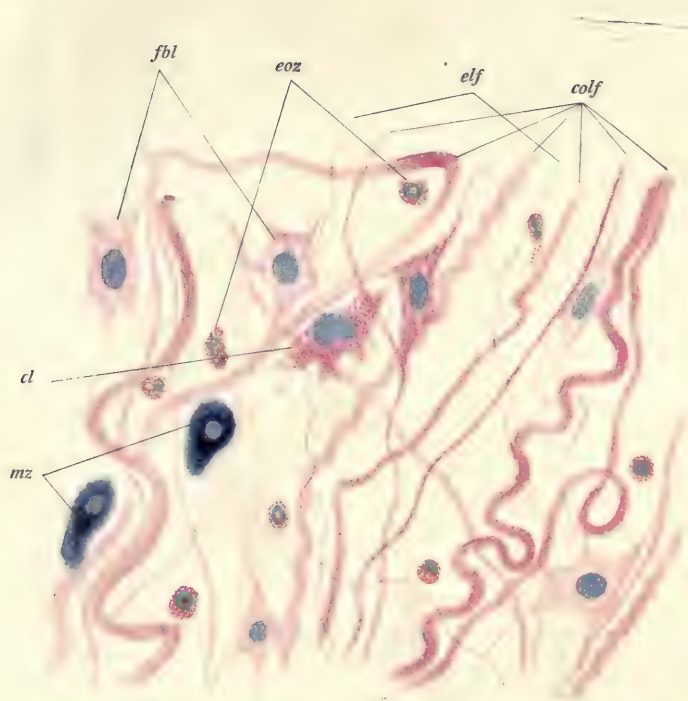


Fig. 36.

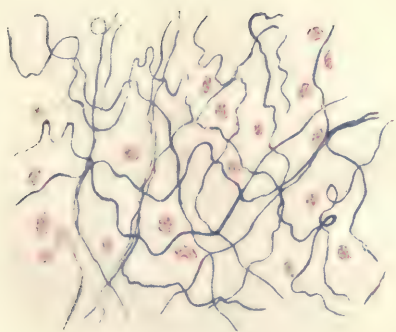


Fig. 37.



Fig. 38.



ing of the collagenous fibres is noticed; they become so transparent as to escape the attention of the superficial observer. From this field the totally unchanged, previously scarcely noticeable elastic fibres stand out prominently. By this reaction, therefore, the latter can always be easily identified.

*The Cellular Element of
Connective Tissue.*

Returning to our original specimen, we will study the cellular element, since little can be learned from unstained specimens in this regard. At first we notice large light-red cells (*fbl*), the bodies of which are provided with long processes, drawn out over wide areas of the specimen. These are the formers of the connective tissue fibres, the **connective tissue corpuscles** or **fibroblasts**. Each cell has a round or oval nucleus. The cellular processes vary in thickness; they are thready or wing-shaped. Very often we can observe how these processes accompany the connective tissue fibres, enveloping them for some distance. The second characteristic cell in our specimen is the **mast-cell** (*mz*). It is round or oval in shape without having any processes, and seldom attains the size of the fibroblast. The characteristic feature of these cells is found in numberless coarse granules, which are scattered throughout them, staining intensely with basic dyes, hence selecting the methyl green of the *Biondi* solution. With it they stain deep blue with a slight tinge of violet. The granules having suffered somewhat by our preliminary treatment, appear slightly swollen. Mast-cells, as seen abundantly in our specimen, are of frequent occurrence in human connective tissue; we will have ample opportunity to meet them in our subsequent studies.

A third type of cells, the *resting migratory cells*, or *clasmatocytes*, are relatively rare in the rat (*cl*). In size they approximate the fibroblasts, likewise being provided with processes, which, however, are always short and stubby. They contain granules, which take up the acid fuchsin from the *Biondi* solution.

Another cell, which is frequently met with in connective tissue, but which does not appear in our picture, is the **migrating cell**, which we met during our study of epithelium. It is derived from the blood or lymphatic system, occurring in varying forms and sizes.

In the rat we frequently encounter **eosinophilic cells** (*eo*), small cells, likewise coming from the blood, having a ring or sausage-shaped nucleus of deep green color. They contain numerous fine orange-red granules.

Fig. 37.—Subcutaneous Connective Tissue from the White Rat

300. Sublimate. Frozen section. Paracarmin. Resorcin-fuchsin.

To enable us to judge the amount of elastic fibres in connective tissue and to demonstrate a specific reaction, we select some frozen sections, place them in 70% alcohol, stain in dilute paracarmin (p. 55) for ten minutes, wash in 70% alcohol and counterstain for fifteen minutes in resorcin-fuchsin (p. 63). After thoroughly washing in 95% alcohol we put them through absolute alcohol and xylol and mount in balsam.

Elastic Fibres.

Our specimen is intersected by numerous thin, winding, elastic fibres, and we are now able to appreciate their great number, which was impossible in the preceding specimen. They are stained deep blue-black, whereas the collagenous fibres, scarcely stained pink, form the background of the field.

Fig. 38.—Cross-Section through the Ligamentum Nuchæ of the Calf

300. Fresh frozen section. Hæmalum.

Elastic fibres are not always as thin as we have seen them in the subcutaneous connective tissue, but may in some places attain a very considerable thickness. As an example we select the posterior neck ligament of the calf, which consists almost entirely of elastic tissue. This can be secured from any slaughter-house. A piece 2–3 mm in thickness is resected with the razor, as near diagonal to the course of the fibre as possible. Frozen sections are made, stained with hæmalum (p. 56), and mounted in balsam.

*Elastic Fibres of
Ligamentum Nuchæ.*

The tissue is made up of closely approximated, yellowish brown fibres, appearing usually round in cross-section and connected among themselves by a light blue intercellular substance. Loose connective tissue divides the entire section into separate bundles. Besides the fibres we also see cells in this tissue, closely approximated to the elastic fibres and having winglike processes. The latter more or less envelope the fibres.

After studying the cross-section we should prepare frozen longitudinal sections, which will show how the separate fibres divide and mingle with others to form a network and, furthermore, how the fibres, by virtue of their elasticity, curl at their cut ends like shepherds' crooks.

PLATE 13

Fig. 39.—Cross-Section through Tendon of Sheep

Fig. 40.—Longitudinal Section through Tendon of Sheep

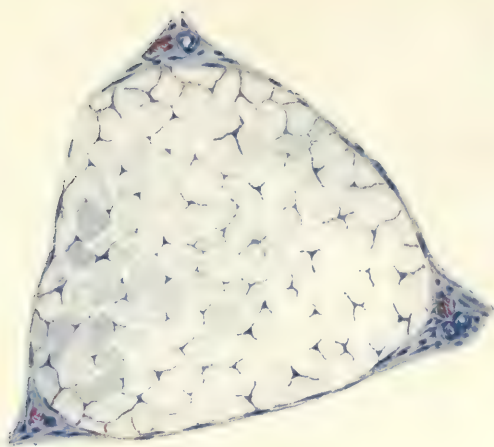


Fig. 39.

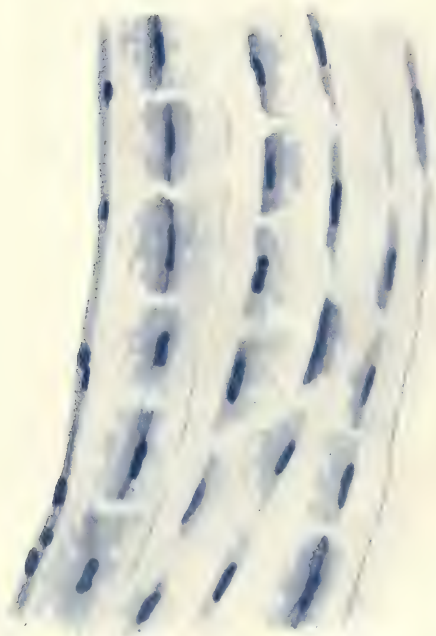


Fig. 40.

Fig. 39.—Cross-Section through Tendon of Sheep

250. $\frac{3}{4}$. Formalin. Frozen section. Hæmalum.

As an example for formed connective tissue we have selected the tendon. The foot of a large animal (sheep, calf) is skinned and fixed *in toto* in 10% formalin. The following day small pieces are cut from the numerous tendons, placed in 5% formalin and after twenty-four hours divided with the freezing microtome into transverse and longitudinal sections. Staining with hæmalum (p. 56). Care should be taken to make the cross-sections really at right angles to the course of the tendon-bundles.

*Formed or Fibrous Connective
Tissue of Tendon;
Peritenonium.*

The cross-section shows the tendon surrounded by a coarse, connective tissue sheath, the *peritenonium*. The latter sends processes into the interior of the tendon, which divide off larger bundles, which in turn consist of smaller bundles, the primary tendon bundles. One of the latter is depicted in Fig. 40, *Tendon fibres*. It is enveloped on all sides by cellular, vascular connective tissue and consists of numerous *tendon fibres*, which are rounded on cross-section, closely approximated, now and then impinging on the calibre of their fellows, so that their cylindrical shapes are here and there changed to prismatic.

Tendon Corpuscles.

Between the fibres stellate cells are seen in close relation with the former, enclosing them in a similar manner as we have seen in the case of the ligament; they are called the **tendon corpuscles**.

Tendon Fibrils.

The tendon fibres, as the loose connective tissue fibres, are again composed of numerous *tendon fibrils*; our transverse section hardly demonstrates this fact.

Fig. 40.—Longitudinal Section through Tendon of Sheep

450. Formalin. Frozen section. Hæmalum.

The picture seen in the cross-section of tendon is materially supplemented by the longitudinal section. Here the tendon fibres are seen to be composed of distinct fibrils. They appear longitudinally striated.

Tendon Corpuscles.

These are seen in various forms, according to whether a transverse or longitudinal section has been made through them. In the former case we will find a flat, approximately rectangular cell-body, emanating from which are seen numerous jagged or thready processes, which may join others to form a network. In the centre of the cell we see a long, rollerlike nucleus. Not infrequently a dark shadow is observed in the axis of the cell, which, upon changing the focus, can be identified as a separate process, leaving the cell-body at right angles. If the cell has been divided longitudinally the elongated nucleus is seen occupying the entire width of the cell. Between these two extremes are those sections where one side of the cell seems closely cut off, the nucleus touching this side.

If we take a mental résumé of all these pictures furnished by the cross-section, we will obtain the following cell-form. Each cell has a thin, elongated body, tightly enveloping the nucleus, flat, winglike processes emanating from the entire length of the cell. These processes grasp and wrap themselves around the neighboring tendon fibres.

The tendon corpuscles are arranged in rows. An interval is always present between two cells.

PLATE 14

**Fig. 41.—Pigmented Connective Tissue Cells from the Pia Mater
of the Sheep**

**Fig. 42.—Fat-Cells from the Subcutaneous Connective Tissue
of the Hedgehog**

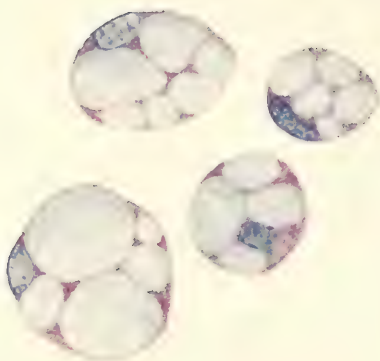


Fig. 42.

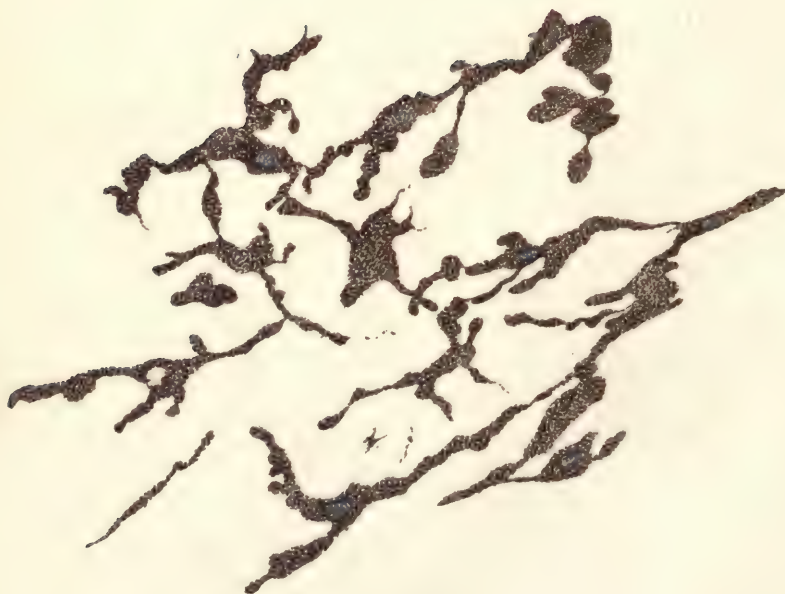


Fig. 41.

Fig. 41.—Pigmented Connective Tissue Cells from the Pia Mater of the Sheep

300. Surface specimen. Formalin. Hæmalum.

In the two following specimens we will acquaint ourselves with some particular properties of the fibroblasts. They are, as we will see, able, in common with epithelial cells, to elaborate in their interior, respectively store up pigment and fat. If pigment or fat cells are present in abundance throughout connective tissue, such tissue is called pigment or fat tissue respectively. Pigment tissue is found in man (at least in the white race) only in the middle layer of the eye, in animals it may be found in other places. A good specimen can easily be secured from the pia mater of the sheep. From the fresh brain, flat sections are taken off the surface with a razor and placed on a wax plate, external surface down. The brain substance can be carefully removed in sections with a scalpel, held vertically, and the remaining meninges fastened with pins. Any remaining brain tissue can be taken off with a camel's-hair brush, moistened in normal saline; the specimen is spread well, fastened with hedgehog bristles and fixed in formalin. The pigmented parts, which can be recognized by the unaided eye, are cut out, stained in hæmalum (p. 56) and mounted in balsam.

Pigmented Fibroblasts.

The pigment cells are fairly large structures having numerous irregular, jagged or thready processes. Their body is filled with minute brown pigment granules. The different cells are connected with others by their processes, thus forming a widely branching pigment net.

Fig. 42.—Fat-Cells from the Subcutaneous Connective Tissue of the Hedgehog

650. *Biondi* solution.

Fat-containing fibroblasts are found in the well-nourished individual, wherever connective tissue develops, principally in the subcutaneous connective tissue. To obtain good specimens, the animal should not be too fat, since an excess of fat will interfere with proper examination. The omentum of young animals or the subcutaneous tissues are adaptable and should be prepared in the manner described on p. II, 46. Still better results are obtained if instead of the saline we directly inject *Biondi* solution (p. 67) with our syringe. After ten to fifteen minutes flat pieces are cut from the plebs, the excess dye is washed out with water, acidulated with acetic acid, and the specimens are mounted in levulose or balsam.

Fat-Cells.

Even with low power the fat-containing places are distinguished by their light-blue color in a red background of connective tissue. The more or less densely distributed fat-cells are of varying sizes, and by continuous storing up of fat will gradually attain a considerable size. The fat is deposited in the form of globules, slightly taking on the basic dye. Between the fat-globules thin septa of reddish protoplasm are seen, which latter also encloses the entire structure as a thin membrane. The smaller drops of fat become confluent, to form larger globules, an immense drop of fat gradually filling the entire cell-body.

The nucleus is always situated close to the cell-wall and appears flattened by the steady pressure of the increasing amount of fat. It may even push the cell-wall outward, so that the cell will assume the shape of a signet-ring, after dissolving out the fat.

PLATE 15

**Fig. 43.—Reticular Connective Tissue, from a Lymph-Gland
of the Cat**

**Fig. 44.—Vesicular Supporting Tissue, from the Sesamoid Bone
of the Tendo Achillis of the Frog**

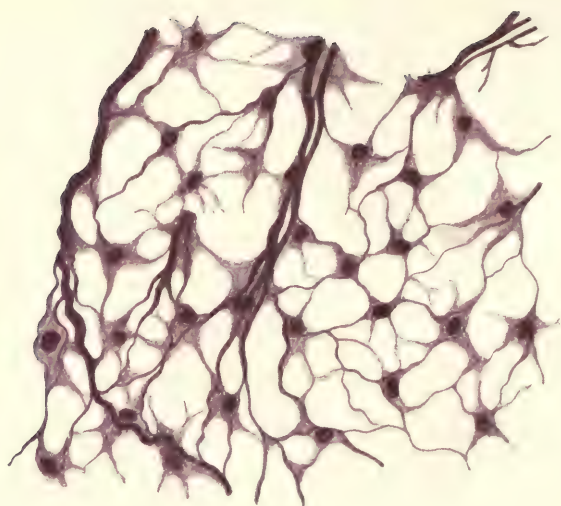


Fig. 43.

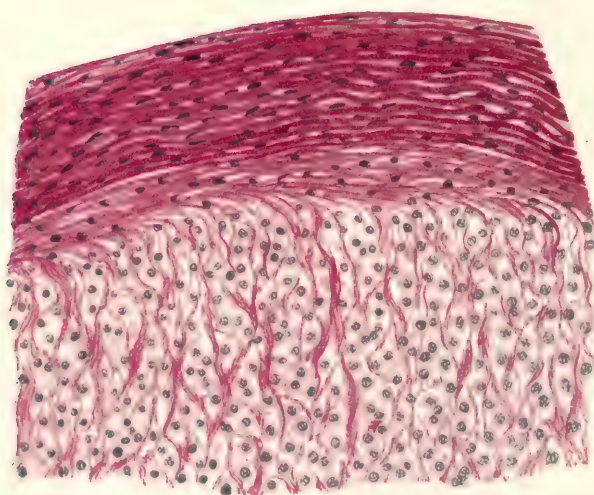


Fig. 44.

Fig. 43.—Reticular Connective Tissue, from a Lymph-Gland of the Cat

600. $\frac{3}{4}$. Formalin. Frozen section. Iodine-iodine-potassium. Gold chloride.

Another distinct form of connective tissue is the reticular variety, found in wide distribution as a basic substance of all lymphoid organs. We have selected the lymph-gland of the cat, e.g., a lymph-gland of the neck; it is fixed in 10% formalin solution, transferred in twenty-four hours into 5% formalin, and frozen sections are made, which are stained after the iodine-iodine-potassium (*Gram's*)-gold chloride method (p. 73) and mounted in levulose or balsam.

Reticular Connective Tissue.

We look for a place in our specimen where the specific element of the lymph-gland, the lymph-cells, are present in small numbers only. Such places, which can be made out with low power by their lighter color, are found directly under the surface, but also in the centre of the specimen, the medulla, so-called. The lymph-cells may also be removed by taking some sections before the gilding process, placing them in a test-tube with a little water and shaking carefully.

High power will show the support of the lymph-glands to consist of a network or reticulum of stellate cells having numerous processes. Connective tissue fibres are also found in varying amounts. In some places the latter will form strong bundles, from which fibres branch off in more or less acute angles constantly, until the entire bundle has dissolved. On other places the fibres are but very minute and scarce. Changing our focus repeatedly will convince us that these fibres cross through the cells everywhere, and in all probability are covered by the protoplasmic processes of those cells.

We may therefore consider reticular tissue as a pre-stage of loose connective tissue, which is derived in its earlier stages of development from a network of anastomosing cells, which in their interior manufacture collagenous fibres. Whereas in the latter variety the fibres become independent from their mother-cells to a greater or lesser degree, they always remain in intimate relation with their cells in reticular connective tissue.

Fig. 44.—Vesicular Supporting Tissue, from the Sesamoid Bone of the Tendo Achillis of the Frog

150. $\frac{3}{4}$. Formalin. Frozen section. *Biondi* solution.

In conclusion, we will mention a transitional subvariety of connective tissue, which will lead us to cartilage, having decided physical similarities to

the latter. The vesicular supporting tissue¹ is seldom found in man, more often as a deposit in tendons of lower vertebrates. The tendo Achillis of the frog will serve well for illustration. Where it crosses the tarso-crural articulation to join the plantar aponeurosis, a considerable thickening can be noticed: the sesamoid bone. The posterior extremity of a freshly killed animal is skinned and amputated at the thigh; we place the specimen in 10% formalin, dissect out the sesamoid bone with a piece of tendon on the following day, place this in 5% formalin. Longitudinal sections are made with the freezing microtome, which should include both the tendon and the sesamoid body.

Vesicular Supporting Tissue.

Under low power we notice how the tendon fibres turn at right angles at the border of the sesamoid, the bundles dividing and dispersing throughout the latter. Between the fibres we can see whole nests of large vesicular cells, closely aggregated. Each cell is surrounded by a thin capsule, not discernible in our specimen, which is instrumental in giving the tissue its stability and cartilaginous consistency.

¹ Literal translation.

PLATE 16

Fig. 45.—Costal Cartilage of the Cat

**Fig. 46.—Cartilage Cells from the Costal Cartilage of a
Young Guinea-Pig**



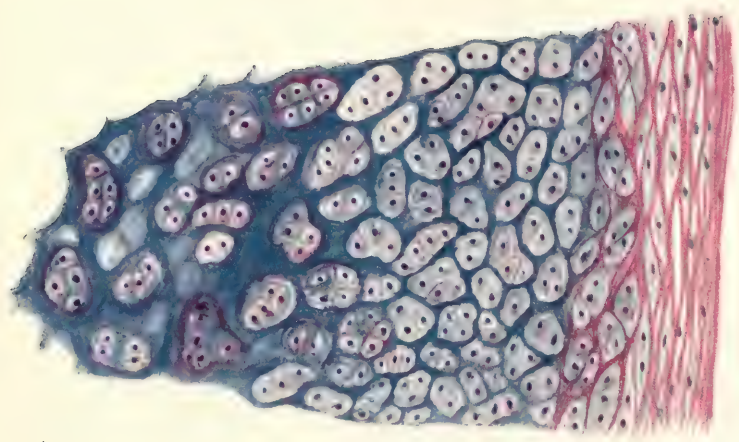


Fig. 45.

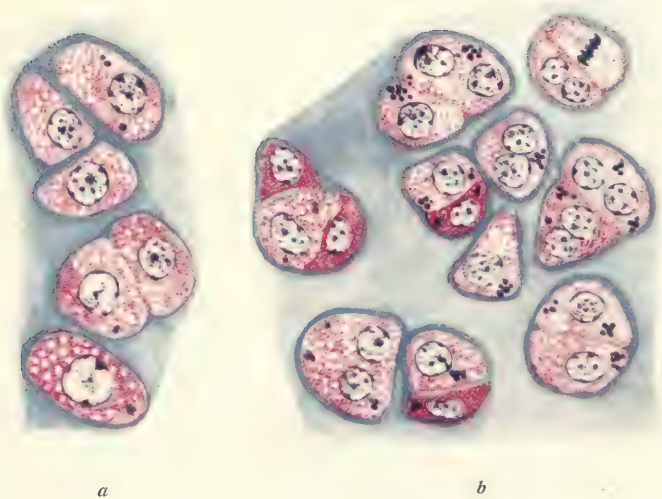


Fig. 46.

3. CARTILAGE

Fig. 45.—Costal Cartilage of the Cat

110. Formalin. Frozen section. *Biondi* solution.

A good representative of hyaline cartilage is found in the costal cartilage of any fairly young mammal. Small pieces are fixed in 10% formalin, transferred on the following day to 5% formalin, and the frozen sections are stained in *Biondi* solution (p. 67).

We can observe macroscopically that the bulk of the section has taken up the basic dye, the periphery alone appearing red. Viewed under low power, the latter is seen to consist of red connective tissue fibres, between which cells are seen, gradually becoming more numerous as they approach the cartilage proper. This membrane, dividing the cartilage from the surrounding connective tissue, has received the name of **perichondrium**.

Further inward we find the actual cartilage; the **basic substance**, staining intensely blue, contains the **cartilage cells**. The latter are situated in hollows of the former, the **cartilage spaces**,¹ which often contain more than one cell. The cartilage spaces are more densely distributed in the peripheral strata than in the central. They are more elongated in the former, attempting to run parallel to the periphery of the cartilage. Toward the centre the cartilage spaces become more irregular.

Fig. 46.—Cartilage Cells from the Costal Cartilage of a Young Guinea-Pig

600. Chromic-osmic-acetic acid. Frozen section. *Biondi* solution.

We will now proceed to study the two main elements of hyaline cartilage on separate specimens. Again we select the costal cartilage of a young animal, e.g., a six to eight weeks' old guinea-pig. Small particles are fixed for two to three days in chromic-osmic-acetic acid (p. 30), washed for twenty-four hours in running water, and thin frozen sections made of them, which are stained in *Biondi* solution (p. 67).

Cartilage Cells.

Under high power we find medium-sized cells, which in the peripheral layers are mostly single (Fig. 46, *a*), whereas in the central portion they are in groups (Fig. 46, *b*). In well-fixed specimens they should occupy the entire

¹ Knorpelhoehlen.

cartilage space. Their shapes vary, being strongly influenced in the central portions by the adjacent cells, with which they share one space. Wedge, sickle and triangular shapes are common. Very often we meet two cells in one space with their flat surfaces pressed together in biscuit form.

Each cell contains a large, mostly round nucleus with scanty chromatin. Quite often binuclear cells are seen, presenting karyokinetic changes, thus showing that the two nuclei have originally been one, and that here the cell division follows the nuclear changes considerably later. In the upper right-hand corner of our picture we see two cells in one space, one of them containing two nuclei, the other attempting to become binuclear, the nucleus undergoing mitosis and just about reaching the state of the equatorial plate.

Glycogen.

The cell-body is formed of a granular protoplasm, which in many cells contains numerous vacuoles. *Intra vitam*, these vacuoles contain drops of glycogen, which has not been preserved by our method. If we want to demonstrate it, we must fix small particles of cartilage in absolute alcohol and examine fine razor sections with a drop of *Gram's* solution (p. 59). The cells will then show a mahogany brown granulation.

Fat.

Besides glycogen we will also find fat in cartilage cells, at least in younger animals, whereas in older specimens it disappears. In our section it has been blackened by the osmic acid, smaller or coarser granules being seen in the cells, generally clumped together in small masses. It is probable that glycogen and fat represent two potent factors in the nourishment of the cartilage cells.

We may draw attention to the fact that in many spaces one cell-body will stain much more intensively than others; evidently the protoplasm in such cells has been rendered more dense.

About the basic substance we cannot learn much from this specimen; it appears evenly stained in a bluish green color. The circumference of each cartilage space seems framed in a zone of intensified color.

PLATE 17

Fig. 47.—Hyaline Cartilage from Calf's Foot

**Fig. 48.—Longitudinal Section through the Ligamentum Teres
Femoris of the Dog**

Fig. 49.—Section through the Cartilage of a Horse's Ear

Fig. 47.—Hyaline Cartilage from Calf's Foot

600. Alcohol-formalin. Frozen section. Methylene blue—picrofuchsin.

To study the basic substance of hyaline cartilage we fix a small piece of the cartilage of a fresh calf's foot for twenty-four hours in alcohol-formalin (p. 34). Thereafter it is kept for an equal length of time in 5% formalin. Frozen thin sections are stained for ten minutes in a 1% solution of methylene blue. After rinsing them thoroughly in water, they are placed in picrofuchsin (p. 66) for two minutes, rinsed in 70% alcohol, dehydrated in absolute alcohol and after xylol mounted in balsam.

The Basic Substance of Hyaline Cartilage.

Under low power the greatest part of the section appears stained red by the acid dye, being divided into numerous **territories**, which enclose the cartilage cells. We select one such cell territory and examine it under high power. In its interior lie the cartilage cells, stained yellow by the picric acid. Some of the cells have retracted from the wall of their space, probably due to the shrinking action of the fixative. Externally the cells are surrounded by a narrow band of especially deep blue basic substance, which also penetrates between the single cells, dividing them more or less completely from their fellows. This band is spoken of as **cartilage capsule**; it may be distinguished even in the fresh cartilage specimen under favorable circumstances from the remaining basic substance by its stronger refraction of the rays of light.

More externally we see a much larger, broader, light blue court, the **inner cell-areola**. This, in turn, is surrounded by a still broader, bright red zone, the **outer cell-areola**. The two areolæ may also be recognized in the fresh specimen, if circumstances are favorable, since their refractive power is much less than that of the capsule, but on the other hand greater than that of the surrounding basic substance. The areolæ rarely enclose but one cell; the rule is that they surround an entire territory.

The remaining basic substance surrounds the areolæ in form of a mesh-work, which has taken a faint bluish red (the hue has not been reproduced entirely accurate). It has been named the **framework** or the **interterritorial substance**; in the fresh specimen it is distinguished by the least refractive power, separating the different cell territories by narrow, sometimes very minute, bands.

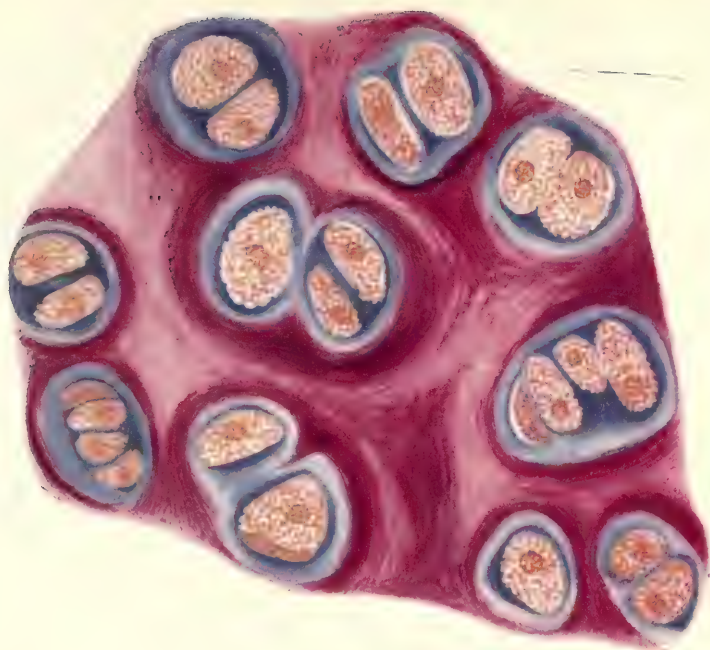


Fig. 47.

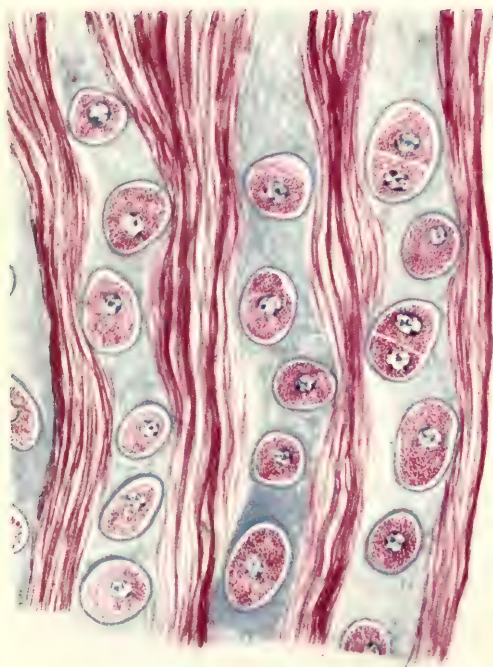


Fig. 48.



Fig 49.

Fig. 48.—Longitudinal Section through the Ligamentum Teres Femoris of the Dog

600. Formalin. Frozen section. *Biondi* solution.

Fibro-cartilage is not found in very many parts of the body of the mammal. We choose the ligamentum teres femoris of a larger mammal or of man, which is easily obtained by opening the capsule of the hip-joint. We see the short band running from the head of the femur to the acetabulum. In cutting it we include on both ends a bit of the articular cartilage. We fix in formalin, make longitudinal sections on the freezing microtome, and stain them with *Biondi* solution (p. 67).

Fibro-Cartilage.

Under low power the band appears to be composed of parallel fibrous connective tissue. Between the **bundles of fibres** we see smaller or larger groups of cells, successively arranged in rows. In the centre of the band few cells are seen, two or three in a group; as we approach the articular cartilage they become more numerous, finally arranging themselves in beams, so that one connective tissue beam alternates with one cell-beam. The cells are surrounded by cartilaginous basic substance, which becomes more pronounced as it approaches the articular cartilage, and finally merges with the latter. In the centre the small groups of cells are situated in little islands, the cells being surrounded by narrow bands of cartilage. The connective tissue bundles decrease in width toward the articular cartilage in conformity with the increasing cartilage beams, their fibres disengage and are finally lost in the basic substance of the articular cartilage.

Under high power we observe that the cells are identical with those of hyaline cartilage. Single or in pairs, they are resting within a space, the deep greenish blue capsules differing distinctly from the lighter basic substance.

Fig. 49.—Section through the Cartilage of a Horse's Ear

300. Formalin. Frozen section. Resorcin fuchsin. *Biondi* solution.

Elastic cartilage is found in some of the laryngeal cartilages, the Eustachian tube and the cartilage of the external ear. The ear of the horse is stripped of its skin, the cartilage cut in small pieces and fixed in formalin. Very thin frozen sections are placed in 70% alcohol and stained after a few minutes in resorcin fuchsin (p. 63). After a quarter of an hour they are thoroughly washed in 95% alcohol, until no more of the dye is extracted, and stained in *Biondi* solution (p. 67).

Elastic Cartilage.

Low power will reveal a network of blue black *elastic fibres*, crossing the light blue basic substance. In the central part the fibres are thickest, be-

coming thinner toward the periphery, until, at the outermost part, they arrange themselves in a band of fibrils, running parallel to the surface of the cartilage.

The cartilage cells are placed, singly or in pairs, throughout the meshes of the network, surrounded by a distinct capsule of basic substance.

The fibres in the ear of the horse are much thicker than those in man. They become thinner by giving off side branches, which anastomose with neighboring branches, forming a network.

PLATE 18

Fig. 50.—Polished Cross-Section through the Human Humerus

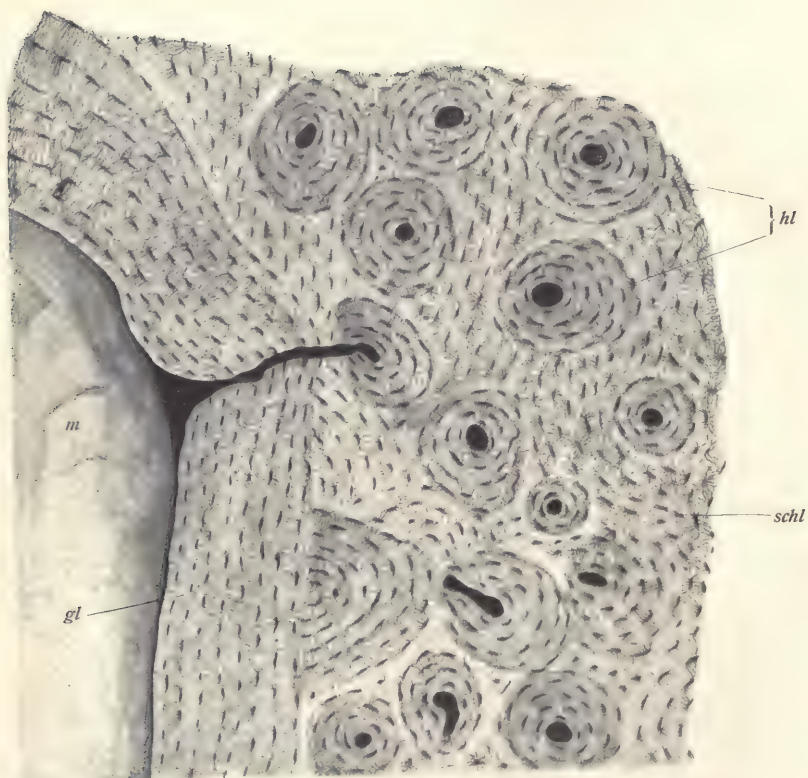


Fig. 50.

4. BONE

Fig. 50.—Polished Cross-Section through the Human Humerus

60. $\frac{3}{4}$.

Preparation of Polished Cross-Section of Bone.

Bony structures are studied in two different ways. We may examine the macerated bone, i.e., that deprived of its soft parts, or, secondly, fixed bone, i.e., containing the soft parts. In order to prepare useful microscopic specimens of a macerated large bone, e.g., the human humerus, we make sections of about 1 mm in thickness with a fret-saw, selecting the diaphysis of the bone and making cuts both diagonal and parallel to the axis. The sawed sections are, of course, much too thick and must be made as thin as paper to be useful under the microscope. The thinning may be accomplished in various ways, the quickest and most perfect being filing. Files of different grain are used, such as the watchmaker employs; they can be purchased in any watchmaker's supply store. Three, or at least two, sizes should be used. A coarse (No. 1 or 2), a medium (No. 3 or 4) and a fine (No. 5 or 6). The medium size may be omitted. Nos. 5 and 6 are rasped on one side only, the other being smooth and serving to polish.

The sawed cut is taken on the tip of a finger and rubbed on the medium or fine file until it is perfectly smooth, the surface showing no more serrations under a low magnification. This smooth surface is polished with the smooth surface of the file. The section is now placed on a warmed slide, covered with a thin layer of cover-glass cement (p. 77), the polished surface being downward against the slide; in a few minutes the cut can be filed down to the thinness of paper. Care must be taken that the section is filed evenly, not thicker on one side than on the other. Furthermore, it must not be made too thin, as it will easily break. It is therefore a good plan to control your work under the microscope from time to time. As soon as it is transparent enough to give a distinct view of the lamellæ and the bone canals with their branches, the filing should be discontinued, the surface polished well and the slide placed in a staining glass containing xylol. If covered and placed in the paraffin-oven, the section will detach itself in the course of half an hour. The specimen is now placed in a vessel of absolute alcohol and cleansed by rubbing between fingers moistened with alcohol. As soon as the alcohol has evaporated, we place the section on a slide and cover it with a cover-glass, using cement to attach the latter. During the cementing a lead weight (bullet) should be placed on top of the cover-glass to prevent curling and twisting of the section.

Haversian Canals.

Fig. 50 shows a small part of such a section, a place near the marrow canal (*m*). We notice immediately numerous obliquely cut canals, appearing black from the air contained within, the **Haversian canals**. Their lumen is either round or oval, on some places drawn out like a handle; the latter is the case at the points of branching, which almost always takes place at an acute angle, as we will learn from the longitudinal section. On one place we can see one such canal disembodying into the marrow-space.

Lamellar Structure of Bone.

The bone substance is arranged concentrically around these canals in a system of lamellæ, forming the **Haversian lamellæ** or **special lamellæ** (*hl*). They may be extensive or very thin, adjacent or separated by inter-spaces, which in turn are filled with bone substance, arranged in lamellæ. This second system of lamellæ does not show any typical course. On various places the lamellæ run in different directions. They are spoken of as **interstitial lamellæ** (*schl*). On the inner surface of the bone, toward the marrow canal, as well as on the outer, toward the periosteum, we find a third system of lamellæ, running parallel to the inner and outer surfaces of the bone, which are called the inner and outer **basic** or **general lamellæ** (*gl*).

Canaliculi.

Aside from the lamellar systems the characteristic appearance of the section is due to the presence of small spaces, the canaliculi, which are also arranged in a distinct order. They are elongated canals, black as the Haversian, but much smaller, which send numerous fine branches into the bone substance, which anastomose among themselves. They lie within the lamellæ, show the same order of arrangement as the latter and are partly instrumental in giving the bony structure its characteristic lamellar appearance, which becomes evident even under low power.

PLATE 19

**Fig. 51.—Longitudinal Polished Section through the Human
Humerus**

Fig. 51.—Longitudinal Polished Section through the Human Humerus

60. $\frac{3}{4}$.

A longitudinal section is prepared in the same manner as the preceding cross-section. The **Haversian canals** (*hk*) appear as long black channels. They branch at an acute angle, thus connecting neighboring canals, giving the longitudinal section the appearance of a network of canals, the meshes running longitudinally. In the same way, as we have seen the canals communicating with the interior surface of the bone, we find it in communication with the outer surface. In our macerated specimen the canals are empty or rather filled with air; in living bone, as we will see later, they are occupied by blood-vessels, conveying nourishment and oxygen to the bone-substance, the vessels entering the canals from the outer as well as inner side of the bone. The Haversian canals thus are important factors in the nutrition of compact bone. Spongy bone, with its paper-thin leaves and plates, has no such canals, because they are not essential for its nourishment.

The longitudinal section likewise shows the lamellar structure of bone distinctly. The canaliculi run parallel to the Haversian canals in the special lamellæ, therefore in longitudinal rows, alternating in the different lamellæ. Between the special lamellæ we can plainly observe the interstitial lamellæ, distinguishable by their irregular arrangement of canaliculi.

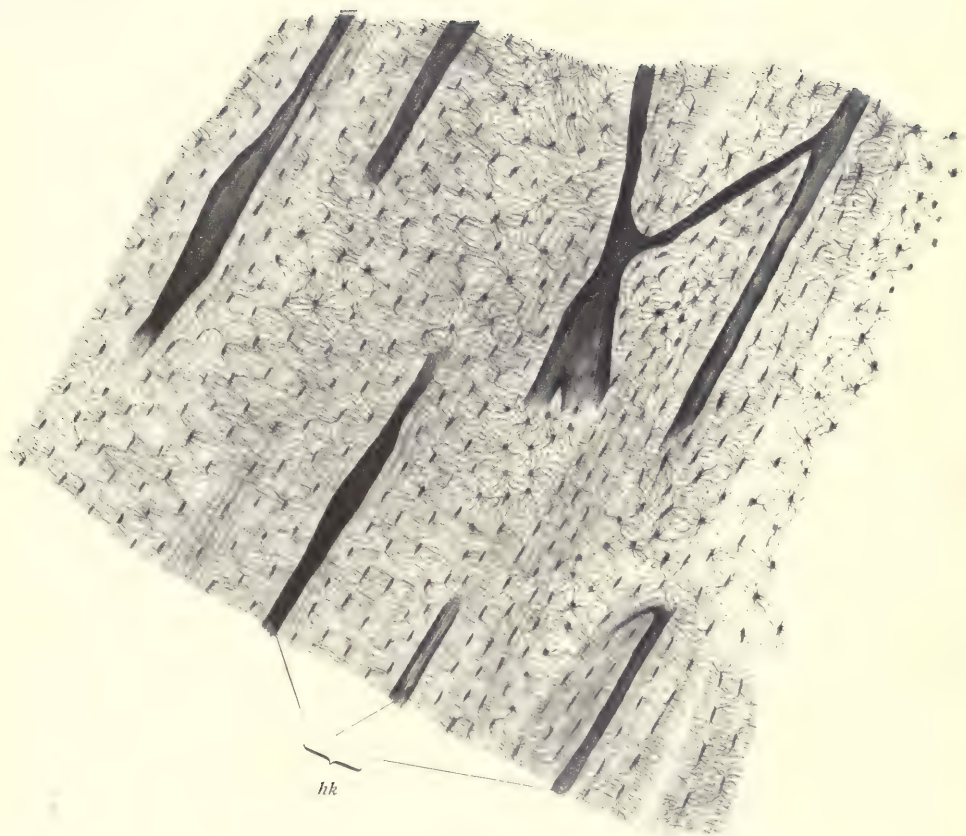


Fig. 51.



PLATE 20

Fig. 52.—Polished Cross-Section through the Human Humerus

Fig. 52.—Polished Cross-Section through the Human Humerus

300. $\frac{3}{4}$. Silver nitrate.

We will endeavor to have a closer view of the bone substance on another specimen. A cross-section is made, in the same manner as described before, of the humerus, transferred from the absolute alcohol to water, wherein it is washed thoroughly. We now place the section in a small amount of a 0.75% solution of silver nitrate. After twenty-four hours the cut will present a decided brown color. It is washed well in water and both sides are polished carefully on a glass plate, in order to remove the irregular deposits of silver, which form on the surface. After dehydration in alcohol, the section is put through xylol and mounted in balsam. Such specimens, while lacking in some details, always bring out the system of lamellæ exceedingly clear. The Haversian canals appear filled with deposits and enclosing them we see the Haversian or special lamellæ, arranged so that one dark brown alternates with one of a lighter brown color.

Cement Substance Between the Systems of Lamellæ.

The edge of the entire system is sharply defined by a curved line against the neighboring interstitial lamellæ. Between the two systems we find cement substance, which is easily recognized by its dark brown color.

If we examine the different lamellæ under high power, we will notice a faint, dense, longitudinal striation in the dark brown lamellæ. This explains the composition of the lamella, being made up of numerous collagenous fibrils, which run circular in these lamellæ. In the lighter lamellæ fine dots are visible. Here the fibrils have been bisected, proving their longitudinal course in the bone, parallel to the Haversian canals.

Collagenous Fibrils of the Basic Substance.

Evidently not so many fibrils are impregnated in the latter case, as there are in the darker brown, circularly fibrillated lamellæ. In certain lamellæ no impregnation has taken place. We also note that the lamellæ containing circular fibrils are often thinner than those fibrillated longitudinally.

The canaliculi and their branches here and there appear well filled with silver salts; however, the impregnation is not thorough enough to allow of a detailed study. Only in the most favorable places can we observe that the branches of those canaliculi, situated in the innermost lamellæ, empty directly into the Haversian canals.

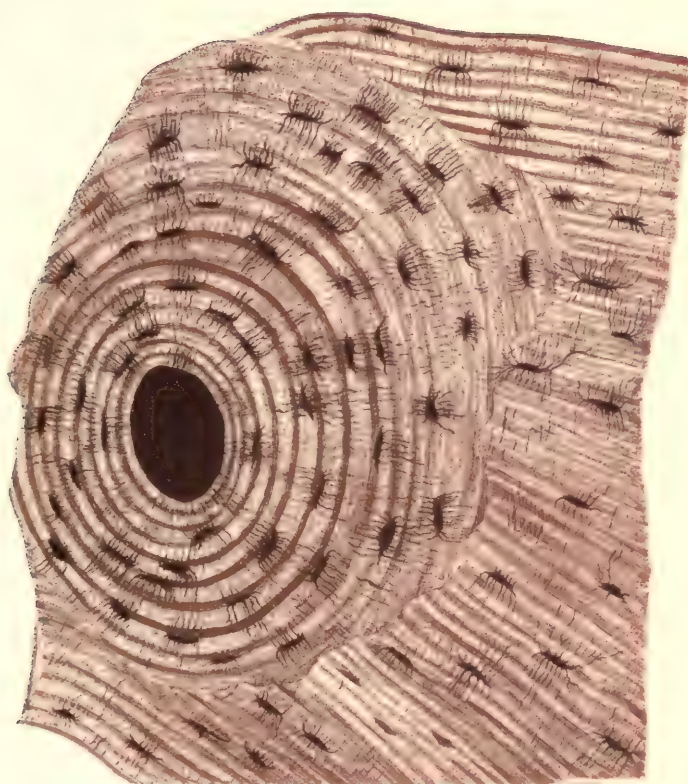


Fig. 52.

PLATE 21

**Figs. 53 and 54.—Canaliculi from Longitudinal and Transverse
Filed Sections of the Human Radius**

**Fig. 55.—Transverse Polished Sections through the Human
Humerus**

**Fig. 56.—Haversian System of Lamellae from a Transverse
Section through the Femur of the Cat**

Figs. 53 and 54.—Canaliculi from Longitudinal and Transverse Filed Sections of the Human Radius

650. Impregnation with acid fuchsin.

The most elegant and convincing specimens, showing the form of the canaliculi and the course of their branches, are without doubt those in which these spaces have been filled with a dye. This can be accomplished in the following manner. A well-macerated bone, e.g., a long bone, is prepared in the same way as described previously; the sections, however, must not be made too thin, the filing being discontinued when they begin to become transparent. They are detached as aforesaid, cleansed in alcohol, dried and placed in a vessel containing 20 cm³ of a 20% solution of acid fuchsin. The container is placed on the screen of a calcium-chloride-exsiccator, which should be provided with tubule and stopcock, such as used in chemical laboratories. The apparatus is connected with a water-pump and evacuation is continued for thirty minutes to one hour. The cock is then closed. After forty-eight hours all water will have evaporated from the vessel, the sections being covered with a thick crust of dry dye. They are carefully removed from the bottom of the dish, the crust is removed with a medium file on both sides and the section filed down with a fine file. For this purpose it must again be mounted on a slide with cement; a control should be frequently made under the microscope. After detaching it with warm xylol, the section should be mounted in Canada balsam.

Canaliculi.

In such sections the channels, having been filled with dyestuffs by the evacuation process, are brought out wonderfully well. In Figs. 53 and 54 some such canaliculi from both longitudinal and cross-sections are represented. Combining both pictures we find that the canaliculi are elongated and irregular and have but a small calibre. They are generally much longer than broad or thick, their longest diameter being parallel to the Haversian canals.

The canaliculi send out numerous branch-channels, which not only anastomose among themselves, but often traverse the lamellæ for a long distance, to finally communicate with a remote canaliculus. In Fig. 54 the mouths of the branch-channels in the canaliculi appear as thick dark red dots.

By their communication interiorly with the Haversian canals and the marrow canal and exteriorly with the periosteum, the canaliculi form a system of channels, which traverse the entire bone and are of the utmost importance for its nutrition.

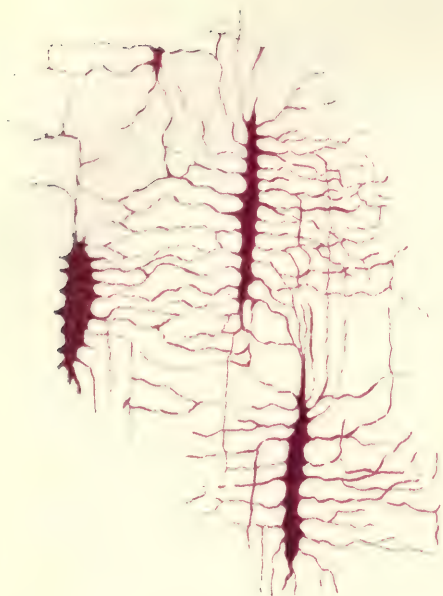


Fig. 53.



Fig. 54.



Fig. 55.

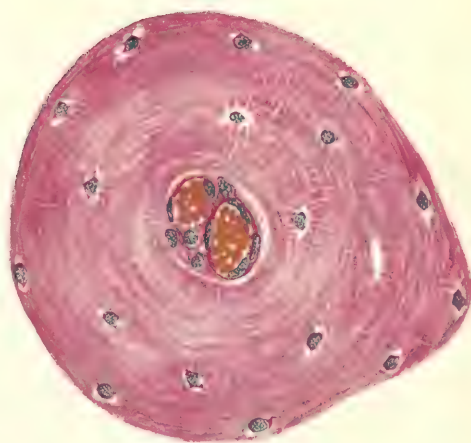


Fig. 56.

Fig. 55.—Transverse Polished Sections through the Human Humerus

600. $\frac{3}{4}$. Gram's solution—gold chloride.

Gilded sections will reveal further structural details of bone. The preparation is the same as in Fig. 52, excepting that we use the iodine-iodine-potassium-gold chloride method (p. 73) instead of the silver nitrate. After gilding, the specimens are again carefully filed on a glass plate, since frequently, especially when the section has not been polished well, deposits of gold will form on the coarse surface. After dehydration the specimens can be mounted in balsam.

Bounding Tubules.

Our specimen gives a most excellent coloring of the canaliculi and their branches, and we notice furthermore that both appear surrounded by a violet membrane. Such membranes have been called **bounding tubules**, and may be compared with cartilage capsules. Like the latter, they are modified, non-fibrillated basic substance, which may be isolated with acids. The course of the canaliculi being tortuous, our section shows the bounding tubules only for a short distance at a time, so that the canaliculi appear light and hollow, alternating with dark and solid. A very pretty illustration is given in these gilded sections of the disembogement of the canaliculi into the Haversian canal.

Fig. 56.—Haversian System of Lamellae from a Transverse Section through the Femur of the Cat

900. $\frac{3}{4}$. Chromic-osmic-acetic acid. Frozen section. *Biondi's* solution.

In order to make cutting sections of bone, the calcium salts must be removed, i.e., the bone must be decalcified. On the well-fixed specimen decalcification may take place in the manner described on p. 48. Or (in smaller objects only) the lime may be extracted by the process of fixation alone. The best solution for this purpose is the combination of chromic-, osmic- and acetic acid (p. 30). Used for small objects, its decalcifying action is very conservative. The thigh of a young cat is stripped of muscle and the femur sawed in two at about the centre with a fret-saw. Sections of 1–2 mm thickness are made with the saw and placed in 25 cm³ of chromic-osmic-acetic acid. The solution is renewed the following day and on the fourth day the sections are washed in running water. If the fixing solution has been shaken frequently, the decalcification will have taken place on the surface to such an extent that a large amount of sections can be made at this juncture. From the water the specimens are directly placed on the freezing microtome. Sections should be made as thin as possible and be stained in *Biondi's* solution.

All soft parts will be preserved by this preparation. The Haversian canals appear filled completely with blood-vessels and small amounts of connective tissue. The Haversian systems of lamellæ are very distinct and the light blue cement lines are quite prominent.

Bone Cells.

Our picture shows a single lamellar system. In the canaliculi we can immediately distinguish the green nuclei of the bone corpuscles. The cell-body is harder to recognize, but by using the immersion-lens and with a good light we are able to make out a small red protoplasma-body around the nucleus, distinct processes radiating toward the branch-canals. We must grant that the processes are never as numerous as these canals and cannot be traced very far into the latter, but we may safely assume that *intra vitam* they are more numerous and their course more protracted.

PLATE 22

**Fig. 57.—Smooth Muscle Fibres from the Urinary Bladder
of the Frog**

**Fig. 58.—Smooth Muscle Fibres from the Urinary Bladder
of the Frog**



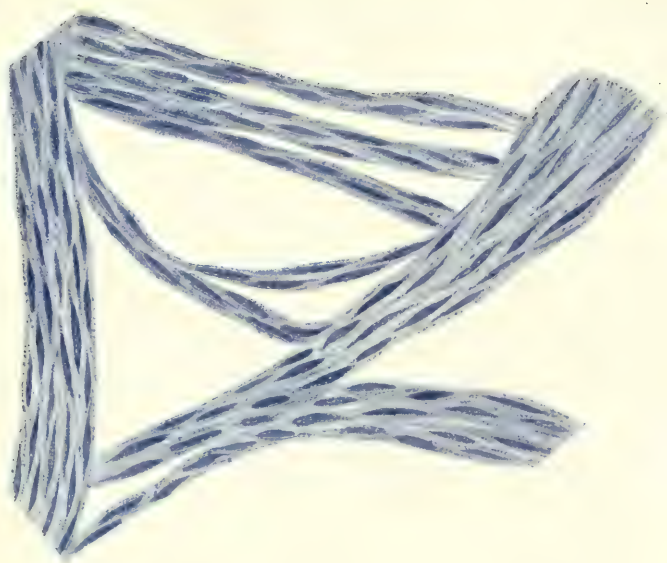


Fig. 57.



Fig. 58.

5. MUSCLE

Fig. 57.—Smooth¹ Muscle Fibres from the Urinary Bladder of the Frog

300. $\frac{3}{4}$. Surface specimen. Hæmalum.

An excellent object for the study of smooth muscle is the urinary bladder of the frog. The abdomen of the decapitated animal is opened, the bladder excised and placed on a wax plate. The viscus is split longitudinally and the bladder wall, slightly stretched, is spread over and fastened on the plate with bristles. Using a pointed scalpel, we cut a window in the plate from below, taking care not to injure our specimen. In this fashion we have obtained an entirely transparent specimen, on which we can observe a network of strands, composed of muscle fibres. We can make our slide still more demonstrative by rinsing the specimens in hæmalum (p. 56) for from ten to fifteen minutes, washing in water and mounting small pieces in levulose, the external bladder surface being uppermost.

Smooth Muscle.

The bladder muscles are arranged in light blue strands, mingling in the form of a net and leaving meshes free from muscle. The width of the strands differs widely. Low power shows long, rollerlike, large nuclei, the ends of which are often pointed, lying in rows. According to the thickness of the muscle strand two or more rows are seen side by side.

Sarcoplasm.

Looking at our specimen with high power, we see that each strand is made up of cells, arranged side by side and one behind another. The different cells can be distinctly seen, being separated by lines of a lighter color. The cell-body is spindle-shaped, ending at either side in a pointed extremity. It stains evenly blue. Only on the periphery of the cell and in its centre we find deep blue, small granules. Those in the centre are in relation with the ends of the nucleus, becoming scarcer as they leave the nucleus, thus appearing much like a continuation of the latter. The nucleus thus appears much more pointed than it really is. This granular substance, the remainder of the former cell protoplasm, has been called the **sarcoplasm**. It takes up the hæmalum eagerly. The entire remaining portion of the cell-body, staining in an even blue, is filled with the contractile substance. Sarcoplasm in the smooth muscle is thus found in the axis of the cell, and secondly closely under the cell surface.

¹ Unstriated.

Fig. 58.—Smooth Muscle Fibres from the Urinary Bladder of the Frog

300. Surface specimen. Silver nitrate. Hæmalum.

To study the outlines and arrangement of smooth muscle cells in detail, we prepare a second specimen in the same manner as before, but place it first in a 1% solution of silver nitrate for five minutes. The bladder is spread on the glass plate, its interior surface upward, and, using a damp camel's-hair brush, we carefully remove the epithelium. The specimen is again placed in the silver solution, this time for thirty minutes, washed thoroughly in water and exposed to the light. On the following day we stain with hæmalum (p. 56) and mount in levulose or balsam.

Cement Substance of the Smooth Muscle Cells.

The muscular bundles appear light yellow and slightly shrunken, due to the action of the silver nitrate. Fine, deep brown to black lines are seen separating the various cells. They are due to a deposit of silver in the intercellular substance, which during life connects the cells, and, of course, give us a clear outline of the cell-body. The cells are cemented so tightly that they cannot be separated by mechanical force; however, the intercellular substance may be dissolved by chemical agents, e.g., 32.5% potassium hydroxide, and the cells isolated thereby.

PLATE 23

**Fig. 59.—Smooth Muscle Fibres from the Urinary Bladder
of the Frog**

**Fig. 60.—Transverse Section through the Muscular Coat of the
Duodenum of a Child**



Fig. 59.

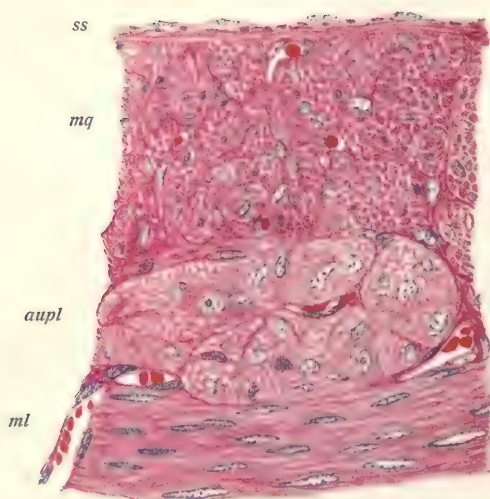


Fig. 60.

Fig. 59.—Smooth Muscle Fibres from the Urinary Bladder of the Frog

1250. Surface specimen. Acetic acid vapors. Sublimate. Iron-alum-hæmatoxylin.

If we desire to demonstrate the contractile substance in our specimen, i.e., stain it, we must take the following measures. The wax plate with the bladder spread as before is placed over a vessel containing acetic acid. After five minutes the bladder epithelium can be completely removed, and the specimen placed for one hour into 2.5% sublimate, washed in running water until the next day and stained with iron-alum-hæmatoxylin (p. 57). During all this time the specimen remains on the wax plate, into which a window has been cut after the reduction. The reduction must be watched under the microscope. Not until it has been in 95% alcohol is the specimen detached from the plate.

Contractile Fibres of the Smooth Muscle Cells.

In favorable places we can recognize a distinct longitudinal striation. The entire cell-body is filled with very delicate longitudinal fibrils. Sarco-plasm cannot be seen. The nucleus frequently shows a very pretty chromatin structure. In our picture the left end of both cells has been cut off.

Fig. 60.—Transverse Section through the Muscular Coat of the Duodenum of a Child

600. Formalin. *Biondi* solution.

The fresh gut is opened and mounted immediately on a wax plate with needles, mucous surface upward. The mucosa is removed by carefully scraping with a scalpel. The specimen is placed in 10% formalin for twenty-four hours and an equal length of time in a 5% solution of the same. Sections of 2–3 mm thickness are made with the razor, placed upon the freezing microtome on their cut surfaces and divided into thin sections. They are stained either in *Biondi* solution (p. 67) or with the iron-alum-hæmatoxylin method (p. 57).

Smooth Muscle Cells in Longitudinal and Cross-Section.

Fig. 60 shows a part of such a section. From without inward we have first one single layer of cells, the serosa or serous coat, which is separated by

connective tissue, the subserosa (*ss*), from the longitudinal muscular layer of the intestine (*mq*). The muscle fibres composing the latter are, of course, cut transversely and appear as small circles, filled with delicate bright red dots, the fibrils. Only now and then we see among the fibrils the cross-section of a nucleus, due to the small size of the nucleus as compared with the length of the cell. Groups of cells are enclosed in connective tissue, forming bundles.

Going farther inward we come to a layer of less interest at this time, containing nerve-fibres and nerve-cells (*anpl*), and then strike the circular layer of muscle (*ml*), where the cells have been cut longitudinally. Here, too, the fibrils may be recognized as fine longitudinal striations, but are not so distinct by far as those seen on transverse section. Between the muscle-fibres we can often observe connective tissue fibres.

PLATE 24

**Fig. 61.—Transverse Section through a Muscle Fibre of the
Gastrocnemius of the Cat**

**Fig. 62.—Transverse Section through a Muscle Fibre of the
Gastrocnemius of the Cat**

Fig. 61.—Transverse Section through a Muscle Fibre of the Gastrocnemius of the Cat

Fresh chop-method. 800.

We will begin the study of transversely striped (striated, voluntary) muscle by preparing a specimen of fresh muscle. Ordinarily small pieces of muscle are macerated with needles, and an indifferent liquid is added. Much more conservative and better for demonstration are those specimens prepared by the chop-method, since besides the longitudinal it also furnishes cross-sections. The gastrocnemius muscle of a freshly killed cat is cut into small particles parallel to the direction of fibres. One piece is placed on a slide and chopped thoroughly with a razor transversely to the direction of fibres; the cover-glass is adjusted and under light pressure it is sealed with a border of lac to prevent evaporation. In order to avoid drying of the specimen, the entire procedure must occupy but little time; cover-glass and slide must have been previously cleansed.

Longitudinal View of Striated Muscle.

Under low power our specimen will show us numerous thicker or thinner fibres, cut short by the chopping process and all showing a distinct transverse striation. The fibres are framed in a smooth, sometimes baggy, border, and on their surface we see, running transversely, closely approximated transverse striations, low power giving the appearance as if light and dark stripes of equal thicknesses were alternating. High power teaches us that the arrangement of these striations is more complicated and that the striæ are of unequal width. Their order of arrangement will be studied later on the stained specimen. A longitudinal striation can barely be recognized in our section.

Striated Muscle Fibres in Cross-Section.

Aside from the longitudinal view we have many cross-sections (Fig. 61). A fibre in cross-section appears more or less circular. It also has a sharply defined border and in its interior we can observe, even with medium power, granules, more abundant in some fibres than in others. The immersion-lens will furthermore reveal a delicate network. The entire cross-section is filled with a network of polygonal meshes. The threads merge into the border of the fibre. The substance of the fibre is thus divided into a large number of polygonal fields, which have been named after their discoverer **Cohnheim's fields**. The network separating the various fields is the **sarcoplasm**. The

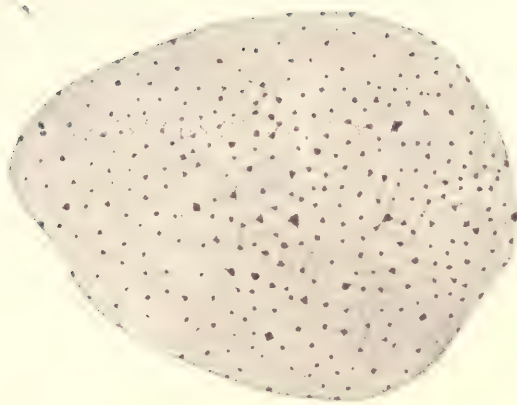


Fig. 61.

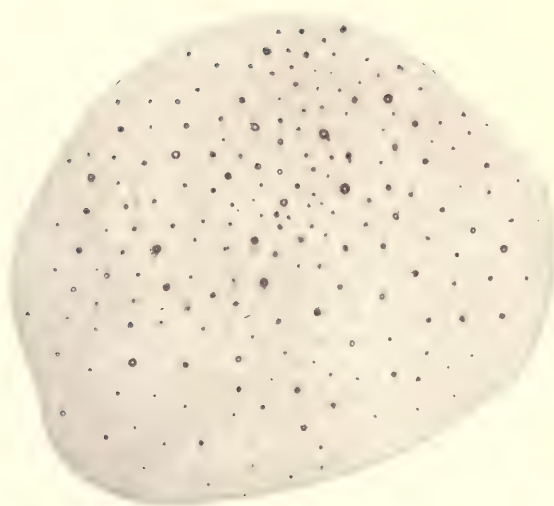


Fig. 62.

latter thus forms a system of thin anastomosing walls, which throughout the muscle fibre enclose prismatic spaces, filled with the contractile substance in form of long tri- to hexahedral columns, the *muscle columns*.¹ The border surrounding the fibre is the *sarcolemma*; it is continuous with the sarcoplasm, appearing like a layer of sarcoplasm surrounding the fibre externally. The entire cross-section of the muscle fibre reminds us of the first specimens of axolotl-liver (p. II, 6), with the exception that here the network is much more regular.

Interstitial Granules.

This comparison becomes still more striking, if we make a close inspection of the granules mentioned previously. Under the immersion-lens they do not appear round, but irregular, always situated at the crossing points of the network; they are also found on the inner aspect of the sarcolemma, so that the beams of sarcoplasm seem to arise from the thickened points of the sarcolemma. They have received the name of *interstitial granules*.

Fig. 62.—Transverse Section through a Muscle Fibre of the Gastrocnemius of the Cat

Fresh chop-specimen. 800. Water.

A second specimen is prepared in the identical manner as before, but the lac border is not drawn around the entire cover-glass, the four corners only being fastened with lac. A similar field as the preceding is searched for, and water is added to the specimen at the edge of the cover-glass with a pipette, until the entire space between cover-glass and slide is filled.

Fat in the Muscle Fibre.

Interesting changes take place now. First the entire design vanishes from the field, due to swelling of the sarcoplasm. Then the interstitial granules swell, become indistinct, shadowlike, and within them a smaller or larger drop appears, which, by its great refraction, we recognize as a *fat drop*.

If the action of the water is continued, the substance in *Cohnheim's* fields will be destroyed, and finally nothing but an empty ring of sarcolemma is left. This phenomenon goes to prove that, although the sarcolemma is originally nothing but sarcoplasm, it has acquired greater resistance toward extrinsic agents than the latter. We may consider it as a modified form of sarcoplasm, to be compared with the ectoplasm of some cells.

If we now extract the water with a strip of tissue paper and substitute a drop of 2% osmic acid, we can observe in some places, where the tissues have been left somewhat intact, the brown or black color of the drops of fat.

Fresh muscle specimens, prepared with the chop-method, are excellent for the study of the action of different reagents on the muscle fibre. Another

¹ Sarcostyles.

very interesting observation can be made after the addition of 32.5% solution of potassium hydroxide, as frequently used for the isolation of muscle fibres. We see a decided shrinking of the entire section, affecting especially the muscle columns, which under the action of this reagent lose their prismatic form entirely and appear like twisted cylinders.

PLATE 25

Fig. 63.—Cross-Section through the Gastrocnemius of the Cat

**Fig. 64.—Cross-Section through the Sterno-Cleido-Mastoid
of the Cat**

**Fig. 65.—Cross-Section through the Rectus Oculi Medialis
of the Cat**



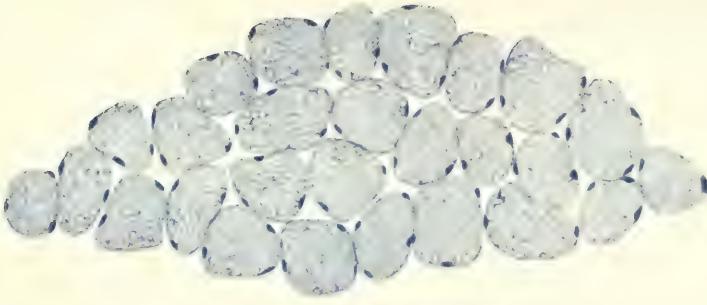


Fig. 63.

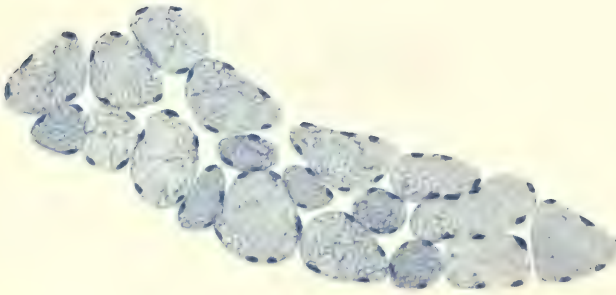


Fig. 64.

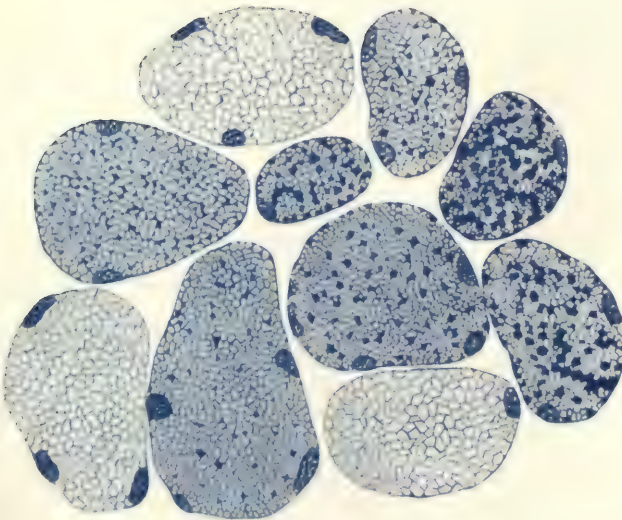


Fig. 65.

Fig. 63.—Cross-Section through the Gastrocnemius of the Cat

150. Frozen section. Hæmalum.

The frozen section will furnish us with a much more distinct cross-section than the chopped specimen. The fresh muscle is cut transversely to the direction of fibres with scissors and placed on its cut surface upon the freezing-table. The specimen is allowed to freeze to the extent of 2–3 mm and cut with the razor at the borderline of the frozen part. Thin frozen sections are made and placed in *Ringer's* fluid (p. 25), stained in hæmalum (p. 56) and mounted in levulose. While the freezing method gives excellent results in general for the study of muscular structure, it shows in this case a few disagreeable attributes. Very often, not always, variously sized spaces are produced among the muscle fibres, probably owing their presence to pre-existing vacuum spaces. However, there are always intact fibres in sufficient amount. Another disadvantage is found in the fact that the muscle fibres do not die from the action of the cold, but retain their contractility, so that, after thawing sets in, the fibre will contract and the section warp.

Frozen Transverse Section of Muscle Fibre.

The cross-section, stained in hæmalum, primarily shows under low power the varying thickness of the fibres, fluctuating within a wide range. The fibres stain evenly blue, distinctly showing the nuclei, which are situated directly under the sarcolemma. Two to three are generally seen in one cross-cut. High power will demonstrate the deep blue network of sarcoplasm with great clearness, merging into the sarcolemma at the periphery. Still darker blue appear the interstitial granules and the nuclei, whereas the muscle columns appear only very light blue.

Fig. 64.—Cross-Section through the Sterno-Cleido-Mastoid of the Cat

150. Frozen section. Hæmalum.

Light and Dark Muscle Fibres.

A different aspect is presented by the sterno-cleido-mastoid of the cat, prepared in the same manner. At the first glance with low power we recognize two different kinds of muscle fibres. One set is identical with those of the gastrocnemius, the others are of an essentially darker blue, and the majority are of smaller size. High power shows that the deeper stain is lim-

ited to the muscle columns, the sarcoplasmic structure being the same as that of the lighter fibres.

Nuclei in the Muscle Fibres.

In the light as well as in the dark muscle fibres we notice a wealth of nuclei. In the cross-section we see double or thrice the amount of nuclei as we observed in the gastrocnemius. The sterno-cleido-mastoid thus is a muscle having both light and dark fibres and being rich in nuclei.

Fig. 65.—Cross-Section through the Rectus Oculi Medialis of the Cat

600. $\frac{3}{4}$. Frozen section. Hæmalum.

*Sarcoplasm Strands and
Fibres Rich in Sarcoplasm.*

Different conditions again prevail in the muscles of the eye. Light and dark fibres are both present; the latter, however, are not always thinner than the former, and distinguish themselves by having a much larger amount of sarcoplasm, which may be present to such an extent as to equal or exceed the amount of contractile substance. We find, therefore, besides the light and dark fibres, sarcoplasm strands and muscular fibres abounding in sarcoplasm. The sarcoplasm often forms beams and strands, which, starting from the sarcolemma, cross through the entire cross-section, more or less.

PLATE 26

**Fig. 66.—Cross-Section through a Fibre of the Semitendinosus
of the Rabbit**

**Fig. 67.—Cross-Section through a Fibre of the Rectus Abdomi-
nalis of the Salamander**

Fig. 68.—Cross-Section through the Gastrocnemius of the Cat

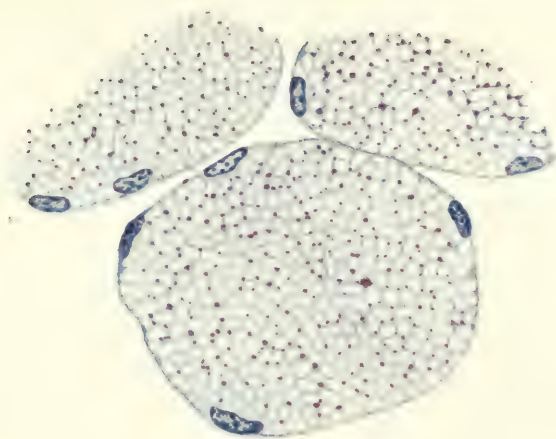


Fig. 68.

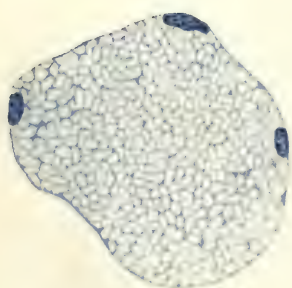


Fig. 66.

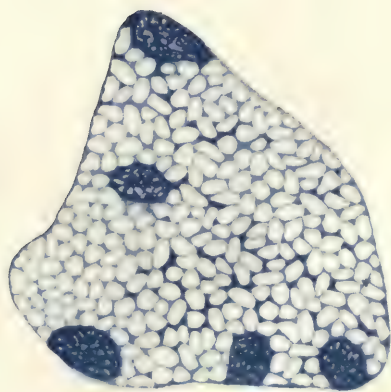


Fig. 67.

Fig. 66.—Cross-Section through a Fibre of the Semitendinosus of the Rabbit

700. $\frac{3}{4}$. Frozen section. Hæmalum.

Red and White Muscle.

We commonly find striking differences in the staining of fresh muscle; some appear very pale, others are decidedly red. We can make this observation especially well on the posterior extremities of the rabbit. On the inner surface we find the adductor magnus, a large and exceptionally pale muscle, and through it we see the slender, deep red semitendinosus. Hence one speaks of red and white muscles; we shall make frozen sections of both these muscles, in order to find any structural differences in their fibres, if such should be present.

Fig. 66 shows a cross-section through a fibre of the semitendinosus. It differs in no way, neither sarcoplasm nor arrangement or size of the sarco-styles, from the adductor magnus. We thus find no difference in structure between red and white muscles. The red color is due to a larger amount and a different arrangement of the blood-vessels.

Fig. 67.—Cross-Section through a Fibre of the Rectus Abdominalis of the Salamander

700. $\frac{3}{4}$. Frozen section. Hæmalum.

We will endeavor to supplement our study of the mammal muscle by investigating the corresponding tissue of an amphibian, the frog or the salamander. The technique is the same as used before, chopped specimen and frozen sections being made.

Muscle Fibres of the Amphibia.

The longitudinal view shows no essential differences. The transverse striation is the same as in the mammal, but the longitudinal striation becomes more distinct in some places. The cross-section proves that in the amphibian as well, the thickness of the fibres varies. The network of sarcoplasm is very similar to that of the mammal, the meshes, however, being generally somewhat larger and hence the muscle columns slightly thicker.

Position of Nuclei.

One essential difference is noted. In the mammal we found peripheral nuclei closely under the sarcolemma; here, however, the same being true of

reptiles and most birds, the nuclei are under the sarcolemma as well as between the muscle columns in the interior of the cross-section. There are muscles in the mammalia having a central nucleus, e.g., the eye muscles, and there it is especially noted in the fibres containing a large amount of sarcoplasm. Now and then we may encounter the same state of affairs in a skeleton muscle, but typically the nuclei of the muscle fibre of mammalia are peripheral.

Fig. 68.—Cross-Section through the Gastrocnemius of the Cat

650. $\frac{3}{4}$. Frozen section. Hæmalum. Sudan.

Interstitial Granules.

In order to make a detailed examination of the interstitial granules, we will prepare a frozen section of the gastrocnemius of the cat, which we stain in the usual manner with hæmalum. After washing the section we take it up on a slide and add 50% alcohol, drop by drop. After it has become quite firm it is transferred to a solution of sudan (p. 65), wherein it is stained from ten to fifteen minutes. We wash in water and mount in levulose.

A very pretty picture results. The crossing points of the sarcoplasm-net, wherein we found the irregular interstitial granules with exclusive hæmalum staining, now contain finer or coarser bright red granules or drops; we can thus corroborate by this stain our previous findings on the fresh specimen: fat drops are found within the interstitial substance. They are partly found as numerous small drops, partly become confluent to form larger drops.

Amount of Fat in the Muscle Fibre.

If different muscles of different animals are examined in this manner, the findings will be that the amount of fat varies greatly in different muscles of the same animal as well as in the same corresponding muscles of different animals, and that many muscles, e.g., of the rabbit, are entirely free of fat. Evidently muscle fat, in contradistinction from cartilage fat, is dependent upon the state of nutrition of the animal.

PLATE 27

**Fig. 69.—Transverse Section through the Rectus Oculi Lateralis
of the Cat**

**Fig. 70.—From a Longitudinal Section through a Muscle
of the Cat**

**Fig. 69.—Transverse Section through the Rectus Oculi Lateralis
of the Cat**

600. Frozen section. Cresyl violet.

A further property of the interstitial granules can be learned by the following procedure. Frozen sections of a muscle, rich in interstitial granules, e.g., an eye muscle of the cat, are stained in a well-diluted solution of cresyl violet (p. 62). After fifteen to twenty minutes we wash in water and mount in levulose.

*Attitude of the Interstitial
Granules toward
Metachromatic Dyes.*

The sections of fibre appear partly faintly red or light blue. In either case the interstitial granules distinguish themselves by a sharply defined deep blue color. On the other hand the network of sarcoplasm is scarcely seen; where it appears, it is always red and of little intensity, as are also the nuclei. This peculiarity explains that the interstitial granules are entirely different in nature from the sarcoplasm. The substance of the muscle columns shows an unstable attitude toward the dye. It stains but faintly, sometimes reddish, other times bluish.

**Fig. 70.—From a Longitudinal Section through a Muscle
of the Cat**

600. Formalin. Frozen section. *Biondi* solution.

As previously explained, it is impossible to make longitudinal frozen sections of the fresh muscle, since the fibres, in thawing, contract so much as to render them utterly useless for examination. We must therefore use fixed material for the longitudinal section. The best solution will be 10% formalin. A muscle of an extremity having parallel fibres should be selected, dissected out and in bulk placed in a sufficient quantity of the solution, so that the muscle is fixed in physiologic extension, viz., without origin or insertion being severed. The next day pieces are cut from the muscle belly, placed into



Fig. 69.

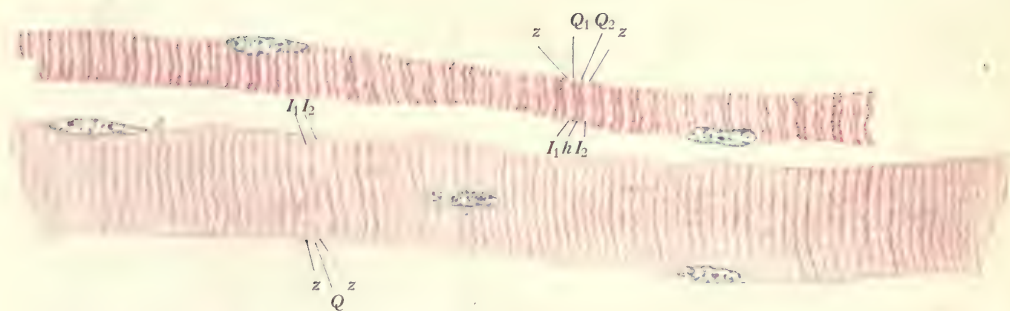


Fig. 70.



5% formalin, and sections are made on the freezing microtome parallel to the direction of fibres. *Biondi* solution is used for staining (p. 67).

The Contractile Substance.

These specimens furnish excellent material for the study of transverse striation. First we go over a specimen with low power, noting the different widths of the fibres and the position of the nuclei, which very frequently appear in the centre of the fibre. Turning to an examination of the striation, we find that two modifications are present in the different fibres. In the simpler case, as shown in the thick fibre of Fig. 70, we see broad red bands crossing the entire width of the fibre in a wavy course, which we name **cross-discs** (*Q*). Between the cross-discs we always find a narrow red band, **the interposed disc**. It is separated from the former by two narrow, very pale red or unstained bands, I_1 and I_2 . We thus have the following order of succession: $ZI_1 QI_2$, etc., in continuous rotation. The whole of these four bands forms a **muscle panel**,¹ so that the fibre is made up of a row of muscle panels. The panels or sarcomeres are of different heights in different animals, both high or low being common.

Examining the single stripe with the highest power available, we recognize that they are not homogeneous, but composed of numerous, closely approximated rods. In *Q* the rods, in the narrow stripe *Z* only the granules are visible. The composition can be recognized in unstained as well as in stained discs, naturally more so in the latter. Expressed in this phenomenon is the fact that the fibre consists of muscle columns (**sarcostyles**), which in turn are composed of still finer **fibrils**. Neither on the fresh cross-section nor on the frozen section can we conclusively prove the composition of the columns of single fibrils, but the pictures furnished by the longitudinal section speak highly in favor of their existence. At any rate we may deduce from previous observation beyond doubt that no cement substance, identical with sarcoplasm, is to be found between the fibrils composing the muscle columns.

Some fibres show the longitudinal striation, which our specimen shows only under high power, quite distinctly even with low power. In the latter case this is due to swelling of the sarcoplasm, which separates the columns, so that we may actually speak of an artifact. Again we may find our longitudinal striation in an immaculate specimen on such places where the interstitial substance is present in large amounts.

Besides the relatively simple variety of transverse striation, described above, we find in other fibres, especially in the thin, more complicated conditions. By sharp observation we will recognize another light line, the **centre disc** (*h*), situated within the cross-disc. This will give us the following successive order in each sarcomere: $ZI_1 Q_1 h Q_2 I_2$. Such fibres usually are made up of higher panels than the simpler fibres. Still further differences are noted in the muscles of different arthropoda, e.g., the yellow and black waterbugs, *dyticus marginalis* and *hydrophilus piceus*. Here each **l-disc** is again divided into two by a darker **para-disc**.

¹ Sarcomere.

If fresh muscle fibres are examined in the polarization-microscope, the stripes *Z* and *Q* (possibly *N*), stained red in our specimen, will appear four times red in a dark field, when the *Nicol* prisms are crossed and the carriage has been turned 360° ; hence they are doubly refractive, ***anisotropic***, *Q* having a stronger anisotropy than *Z* and *N*. The stripes *I* and *h* are simple refractive, ***isotropic***.

PLATE 28

**Fig. 71.—Longitudinal Section through Papillary Muscle of
Monkey**

Fig. 72.—Cross-Section through Papillary Muscle of Monkey

Fig. 73.—Cross-Section through Papillary Muscle of Monkey

Fig. 71.—Longitudinal Section through Papillary Muscle of Monkey

650. $\frac{3}{4}$. Sublimate-acetic acid. Frozen section. *Biondi* solution.

The heart muscle, which we will now consider, offers special structural peculiarities. If good specimens are wanted, the material must be absolutely fresh. The heart of a cat or dog, or preferably that of a monkey, is selected, pieces of the heart-wall being placed for from four to six hours in a fresh 2.5% solution of sublimate, to which 1% of acetic acid has been added. The solution should be warmed to body temperature. (The remainder of the heart is used for the preparation of fresh chop and frozen sections.) After completed fixation we wash in running water for twenty-four hours and transfer to 5% formalin. The following day, thin longitudinal and transverse sections are made, preferably of the papillary muscles. The sections are stained in *Biondi* solution (p. 67) or in iron-alum-hæmatoxylin (p. 57).

Heart Muscle Fibres.

Fig. 71 shows a longitudinal section through a papillary muscle. We notice at once that here we have to do with a network of muscle. The fibres divide, almost always dichotomously, anastomosing among themselves. We thus have a network, in the meshes of which the blood-vessels are situated. While we may find branching muscle fibres in other structures, e.g., the tongue, and muscle fibres arranged in netform still elsewhere, this arrangement is primarily characteristic of heart muscle.

On the average the fibres are thinner than those of the skeleton-muscles; they have a marked transverse striation, illustrating especially the interposed disc, while *Q* and *I* are less distinctly separate. Heart muscle also shows the longitudinal striation very distinctly. The different fibrils can be clearly differentiated.

Another peculiarity of the heart muscle discriminates it from the skeleton muscle absolutely. At shorter or greater distances the fibres are crossed by transverse stripes, which by their strong color immediately attract our attention. They are usually called **interpolated sections** (*tr*). They consist of closely approximated, shorter or longer rods, interpolated in the course of the fibrils, in their entirety forming a band across the muscle fibre. The bands often show flaws, imparting the appearance of *stairs*, which latter name is also in use. In reality we are not dealing with stripes, but with rounded or elongated plates, which traverse the entire thickness of the fibre. Little can be said about the significance of these plates at the present time. The name, interpolated sections, implies that we have plates, which are interposed between short pieces of fibre. We can isolate them by placing small pieces of heart muscle in a 32.5% solution of potassium hydroxide for thirty

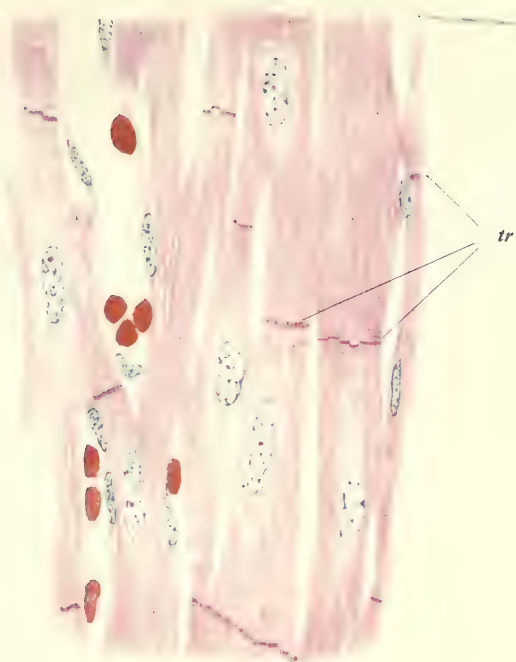


Fig. 71.

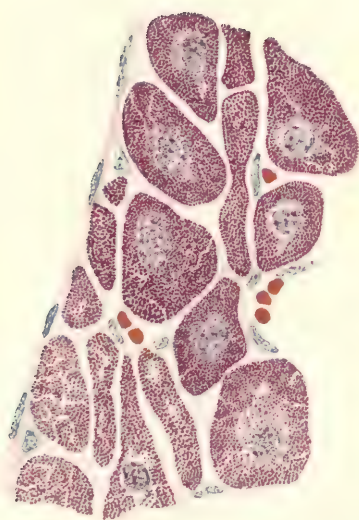


Fig. 72.

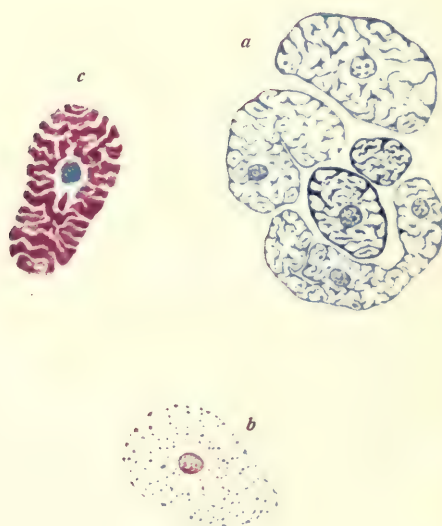


Fig. 73.

minutes, macerating while in the solution. The segments will be found to contain one or more nuclei; they have an irregular form, short stubby branches, and on their ends are provided with serrations, corresponding to the stairs. Such isolated formations are called **heart muscle cells**. Hence we might consider the entire heart muscle as composed of numerous anastomosing cells. Whether these formations should actually receive the value of cells or whether they should be considered as artifacts, is still an open question.

The nuclei of heart muscle are very similar to those of the skeleton muscle. They are always surrounded by a distinct mass of granular sarcoplasm.

Fig. 72.—Cross-Section through Papillary Muscle of Monkey

650. $\frac{3}{4}$. Sublimate-acetic acid. Frozen section. *Biondi* solution.

Cross-sections, made in the same manner as the preceding, show the fibres as irregular, rounded, elongated, triangular or quadrilateral formations, the shape of which has, without doubt, been influenced by our method of fixation. In many places we can even here recognize the anastomosis of fibres. The section will then appear drawn out, or two rounded fibre sections may be seen connected by a narrower bridge.

In the interior of the fibre we usually find a slightly irregular *nucleus*, surrounded by more or less finely granular **sarcoplasm**. The remainder of the bisected fibre is filled out by transversely cut **fibrils**.

Fibrils of the Heart Muscle Fibre.

They appear round, are closely aggregated, still leaving minute light interspaces. Narrow clefts run through the mass of fibrils here and there, communicating either with the periphery or with the circumnuclear sarcoplasm, so that on many places the entire mass of fibrils is split into larger groups. Between the various fibre sections connective tissue fibres, nucleated connective tissue cells and small blood-vessels are seen.

Fig. 73.—Cross-Section through Papillary Muscle of Monkey

650. Frozen section. a, hæmalum; b, cresyl violet; c, *Biondi* solution.

Let us supplement the knowledge of heart muscle, gained so far, by examining the unfixed specimen. For this purpose we will stain thin frozen cross-sections of a fresh papillary muscle first in hæmalum (p. 56), then in cresyl violet (p. 62), and thirdly in *Biondi* solution (p. 67), all to be mounted in levulose.

Sarcoplasm of the Heart Muscle Fibre.

The section stained with hæmalum (Fig. 73, a), similar to that of the skeleton muscle, gives us a very descriptive picture of the structural proper-

ties by coloring all parts of the sarcoplasm blue. Just as we found it in the skeleton muscle, we note that the heart muscle fibre is surrounded by a continuous layer of sarcoplasm, the sarcolemma, from which numerous sarcoplasmic extensions arise and penetrate through the interior of the fibre toward the nucleus. Hence the contractile substance of the heart-muscle fibre is again divided into compartments by the septa of sarcoplasm; the septa, however, do not form a regular meshwork, but simply mingle, so that we lack the regular prismatic sarcostyles and have in their stead leaves of contractile substance, which, anastomosing among themselves, radiate from the periphery toward the interior of the fibre.

An areola of sarcoplasm around the nucleus is absolutely imperceptible, this fact compelling us to consider these circumnuclear masses of protoplasm, shown in the fixed specimen, as artificial products, probably due to shrinking of the contractile substance.

*Interstitial Granules in the
Heart Muscle Fibre.*

The section stained with cresyl violet (Fig. 73, b) is complementary to the preceding. The nucleus has taken a red color as usual. Aside from it we see blue granules in the fibre. They are located primarily on the periphery, but also throughout the cross-section, their arrangement showing that they are situated within the sarcoplasmic septa. In this section we have therefore stained only the interstitial granules, not the sarcoplasm.

*Contractile Substance of the
Heart Muscle Fibre.*

The third section, stained in *Biondi* solution, shows a green nucleus and red contractile substance. Evidently the action of the dye on the frozen section is such as to cause a swelling and dissolution of the sarcoplasm and synchronously a shrinkage of the contractile substance. The beams (sarcostyles), compared to the hæmalum specimen, have shrunk not only longitudinally but also transversely, so that we find an empty space around the nucleus, while the septa are separated by larger clefts.

How may we reconcile this picture with the fixed specimen shown in Fig. 72? In the latter the entire cross-section of a fibre was filled with numerous sections of the fibrils; here we find compact leaves of muscle without a trace of individual fibrils. These leaves evidently correspond to the muscle-columns of the skeleton muscle, since here as well as there they are formations, separated by sarcoplasmic septa. They differ, of course, in shape. A similar structural arrangement of the contractile substance is found in certain skeleton muscles of some fishes. In the fixed specimen the sarcoplasm is destroyed, except a minimum amount, the leaves shrink and split into component parts, which we designate as fibrils. Similar conditions were demonstrated in the cross-section of the fixed striated muscle fibre. It was filled in an irregular manner with shrunken muscle-columns, which we now recognize as fibrils. The original typical picture of *Cohnheim's* field has been almost entirely lost.

PLATE 29

Fig. 74.—Unipolar Nerve-Cells from the Spinal Ganglion of the Rabbit

Fig. 75.—Bipolar Nerve-Cells from the Spiral Ganglion of the Cat

Fig. 76.—Multipolar Nerve-Cells from the Anterior Horn of the Spinal Cord of the Rabbit

6. NERVE TISSUE

Fig. 74.—Unipolar Nerve-Cells from the Spinal Ganglion of the Rabbit

300. Silver-ammonia method. Paraffin section.

Unipolar cells are most easily studied in the spinal ganglia. The spinal canal of a rabbit is opened in the usual manner, the lamina resected with bone forceps enough to expose the intervertebral foramina and the spinal ganglia situated within them. The ganglia are excised, fixed in 10% formalin, and treated with the silver-ammonia method (p. 71). Paraffin sections of 10–15 μ in thickness are made.

A section will show the composition of the ganglion of spherical or pyriform large cells. The cell-body has taken a brown stain, the large vesical-shaped nucleus appearing like a light stain.

Unipolar Nerve-Cells.

On favorable places we can see a strong process arising from the cell-body, which soon after its exit from the cell curls up in numerous contortions, which are closely crowded together, forming a **glomerulus**. The tract can often be traced for a long distance; the details thereof will be discussed in a special part, provided in our course. In some cases the glomerulus-formation is not so pronounced, consisting of but two twists.

The Capsule of the Peripheral Nerve-Cells.

Low power will already show numerous nuclei on the periphery. Under high power we recognize that all the unipolar cells are surrounded by **capsules**, which consist of connective tissue fibres and cells, surrounding the cells on all sides. Interiorly, between capsule and cell-body, numerous cells are situated, to which belong the aforesaid nuclei; these cells have been called **mantle-cells** or **amphicytes**. If we examine the relation of the cellular process to the capsule, we find that the glomerulus proper lies within the capsule, the process later piercing it.

Fig. 75.—Bipolar Nerve-Cells from the Spiral Ganglion of the Cat

300. Chromic-osmic-acetic acid. Paraffin section. Hæmalum.

In the grown mammal and in man bipolar cells are found only in the ganglia of the auditory nerve. Newborn cats or guinea-pigs are most

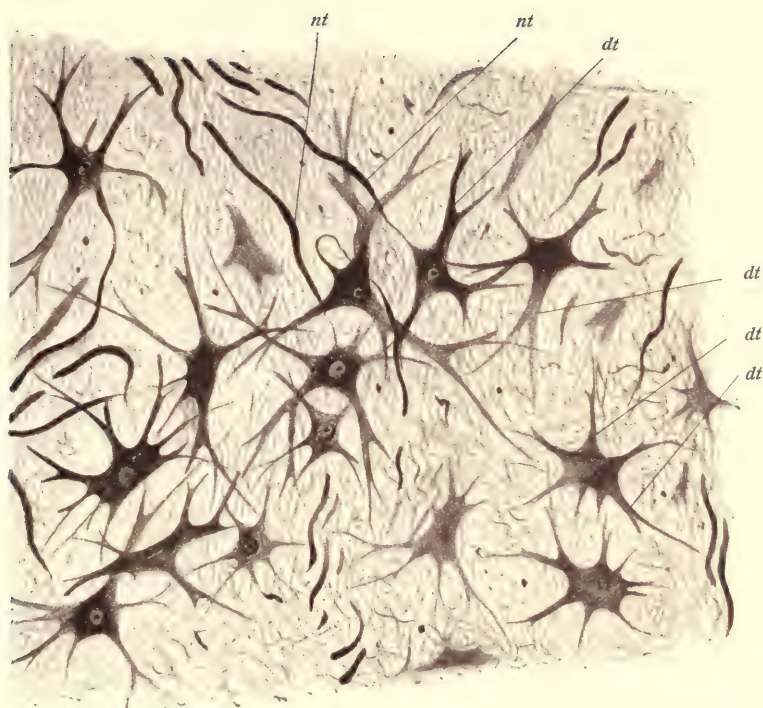


Fig. 76.

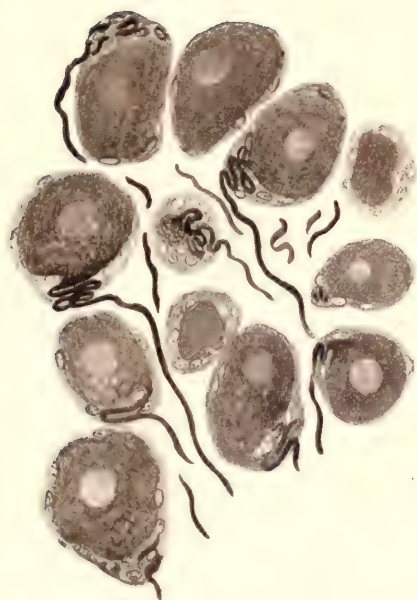


Fig. 74.

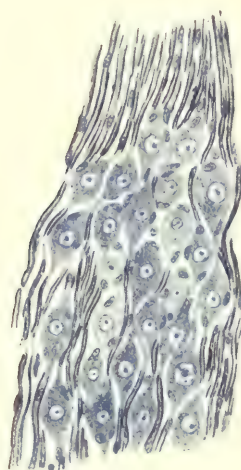


Fig. 75.

suitable for examination. After removing the brain, we disarticulate the lower jaw, and the base of the skull, including the bony labyrinth closely behind the mandible, is laid open. The entire petrous portion of the temporal bone is removed from the skull. The bony labyrinth, cartilaginous as yet, is opened and the specimen, in this state, is suspended in 50 cm³ of chromic-osmic-acetic acid (p. 30) for from three to five days. The liquid should be changed once or twice. The specimen is now washed in running water for twenty-four hours and embedded in paraffin in the usual manner. While in 95% alcohol the cochlea, which can be seen within the labyrinth, is bisected longitudinally, the paraffin sections being made parallel to this plane. The sections are stained in hæmalum (p. 56). Within the cochlea small bundles of nerve-cells are seen, which make up the spiral ganglion.

Bipolar Nerve-Cells.

Under high power we find oval cell-bodies, which are pointed at both ends. Each end is continued as a light blue fibre, which after a short course takes on a black sheath, which will be discussed in detail later. Thus we have one nerve-fibre leaving each pole of the cell, or we may say that the nerve-cell is nothing but a nucleated swelling in the course of a fibre. These cells are likewise surrounded by a capsule, covering amphicytes and nuclei. The nucleus of the nerve-cells is globular, contains little stained chromatin, but an exceptionally large nucleolus. Within the cell-body numerous fine granules are seen, which have been stained intensely with the hæmalum.

Fig. 76.—Multipolar Nerve-Cells from the Anterior Horn of the Spinal Cord of the Rabbit

150. Silver-ammonia method. Paraffin section.

The best developed and more or less largest multipolar cells are found in the spinal cord. The cord is exposed to view and, starting at the cauda equina, is taken from the spinal canal. Pieces, 2-3 mm thick, are cut from the cervical or lumbar region with a razor or sharp scissors, care being taken not to compress the tissue. The pieces are fixed in 10% formalin and treated with the silver-ammonia method (p. 71). The paraffin sections must not be too thin in this case (15-25 μ).

Multipolar Nerve-Cells.

Even with low power we immediately recognize the anterior horn by its wealth in large nerve-cells. They are irregular, most often polygonous structures, the corners of which are drawn out into long processes. Each process arises by a broad base from the cell-body and, constantly tapering, it sends off branches, the terminal branches ending in very fine points (*dt*). These so-called **protoplasmic processes** or **dendrites** can be traced for long distances. Their number varies, and close observation will teach us that their form and manner of origin differ materially. In some other field we may, for instance, find a sort of polar arrangement of the dendrites, the

latter radiating either on one side only or bipolar. The dendrites in our specimen have taken a light brown stain, which becomes lighter as the dendrite splits into branches in its course from the cell.

Besides these dendrites we notice another form of cell process. They are dark brown fibres of unchanging calibre throughout the specimen. They are generally separated from their cells of origin, but in favorable cases (*nt*) we can trace them to the latter. These **neurites**¹ arise, one from each cell, from a corner of the cell-body, similar to the dendrites; the process, in contradistinction to the dendrite, immediately becomes thin, but only for a short space, when it again assumes a thickness, which now remains constant. At first the neurite is very pale, but becomes very dark as soon as it thickens. The neurites do not branch dichotomously, as do the dendrites, but remain undivided. Thus we have on each multipolar cell several branching dendrites and one undivided neurite (or axis-cylinder), this rule not only holding good for the multipolar nerve-cells in the spinal cord but for all multiple cells of the nervous system in general.

*The Central Nerve-Cell
has no Capsule.*

Another important fact is demonstrated in these cells. While the cells of the spinal and the spiral ganglia were incapsulated, we have here neither capsule nor amphicytes. All cells of the central nervous system, brain and cord, are nude, while the cells of the peripheral system, i.e., the cells constituting the cerebrospinal and sympathetic ganglia, are clothed in a capsule.

¹ Axis-cylinders.

PLATE 30

**Fig. 77.—Multipolar Nerve-Cells from the Anterior Horn of the
Spinal Cord of the Rabbit**

Fig. 77.—Multipolar Nerve-Cells from the Anterior Horn of the Spinal Cord of the Rabbit

580. $\frac{3}{4}$. Pyrogallol method. Paraffin section.

After going over the shape and the processes of the nerve-cell, a special part dealing with the varieties of the former, we will proceed to take a closer view of the structural peculiarities. In order to bring out the most important component parts of the cell-body, we treat small particles of the cord of a rabbit with the pyrogallol method, described on p. 71. Paraffin sections, of 5–10 μ thickness, are made and preferably are gilded (p. 71) in addition.

Fibrils in Nerve-Cells.

Such specimens show us the most important part of the nerve-cell, the **neurofibrils**, with exceptional clearness. The dendrites, to begin with, show closely packed strands of delicate fibrils. Coming from all the branches, they are collected by the dendrite trunk, whence they radiate into the cell-body. In the dendrites they are undivided in general, running parallel; only where the dendrite branches we may recognize unmistakable **net-formations**. The fibrils enter the cell-body in bundles, crossing the cell, either to enter another dendrite or the neurite. A neurite is seen on the large cell in the centre of our picture in the form of a process leaving at the left side in a straight course. Besides the strands of fibrils, going directly from dendrite to dendrite and from dendrite to neurite, we have a genuine network of fibrils distributed over the cell-body, which is best developed in the outer strata of the cell. The fibres composing it are partly derived from the dendrites, partly from the neurite. In the latter the neurofibrils are so closely approximated as to make it impossible for us to distinguish them singly.

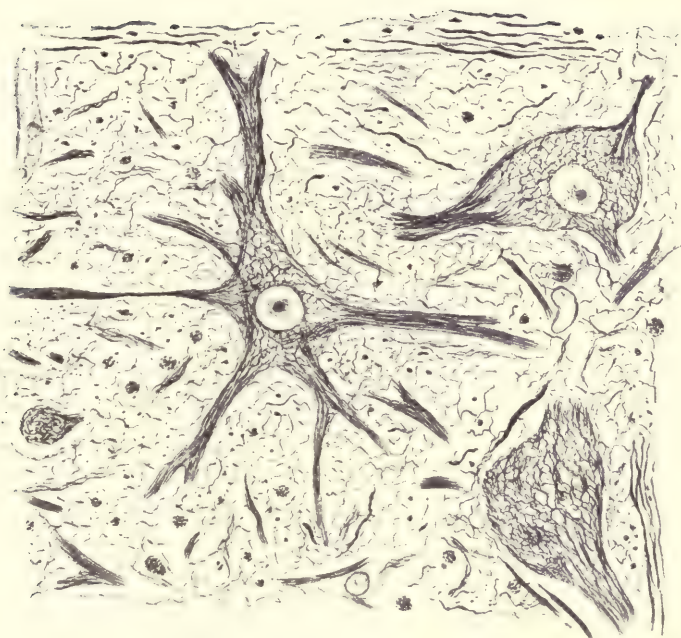


Fig. 77.

PLATE 31

**Fig. 78.—Multipolar Nerve-Cells from the Anterior Horn of the
Spinal Cord of the Rabbit**

Fig. 79.—Nerve-Fibres from the Great Sciatic Nerve of the Frog

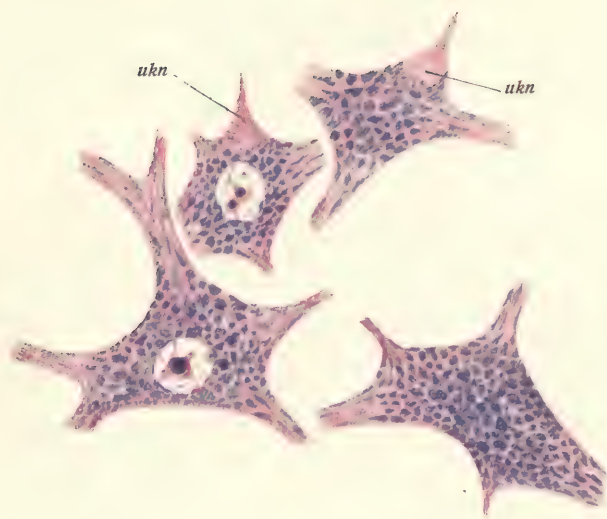


Fig. 78.

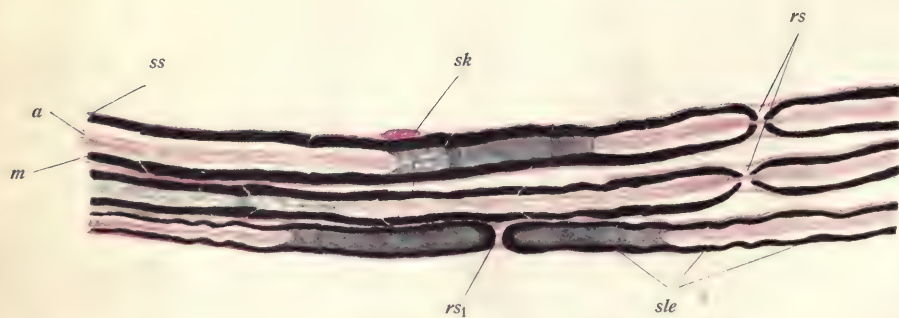


Fig. 79.

Fig. 78.—Multipolar Nerve-Cells from the Anterior Horn of the Spinal Cord of the Rabbit

450. Alcohol-acetic acid; paraffin embedding; methylene blue; acid fuchsin.

We will finally prepare a specimen, which shall inform us about the structure of the remainder of the cell-body and of the nucleus thereof. Small pieces of spinal cord of the rabbit, or any other mammal, are fixed in alcohol-acetic acid, according to the rules given on page 33, and embedded in paraffin. Paraffin sections of 5 μ thickness are stained for fifteen to twenty minutes in a 1% solution of methylene blue, the process taking place in a well-covered glass in the paraffin oven. The dark blue sections are rinsed in water to remove the excess of dye, and thereafter transferred to 95% alcohol, where heavy clouds of dye will be extracted. We counterstain in a 0.1% solution of acid fuchsin until the sections appear pale red, when they are rinsed in 95% alcohol, dehydrated in absolute alcohol, placed in xylol and mounted in balsam.

Basophilic Substance of the Nerve-Cells.

We select again the large multipolar cells of the anterior horn, where we find the bodies studded with numerous intensely blue clods, the **basophilic corpuscles** or **clods of Nissl** (granules of *Nissl*). They appear as irregular, polygonous, serrated clods, being densest in the vicinity of the nucleus; toward the dendrites they become lighter, smaller and appear more elongated, as if adapting their shape to their surroundings. They can be traced into the dendrites for some distance, disappearing in the finer branches. One place, aside from the nucleus, is always free from granules, namely, the cone of origin of the neurite (*ukn*); the latter itself never contains any clods. Viewed under high power, these clods are not homogeneous, but composed of closely arranged fine granules. The term "basophil" applied to these clods only means that they stain well with basic dyes; they are not basophilic in the sense in which we speak of the nuclear chromatin, which we can easily prove by staining such a section with *Biondi* solution, where they will take up the red acid fuchsin, but not the basic dye.

In other nerve-cells these granules vary as to size and arrangement, to wit, in the spinal ganglionic cells, where they are much smaller, and in the sympathetic cells, where they are densely distributed in the outer zone of the cell, but very scanty around the nucleus. We have already encountered these clods in our specimen 75. The fine granules, seen there in the body of the spiral ganglion cell, were nothing but small *Nissl* granules.

*The Nucleus of
the Nerve-Cell.*

As shown in our specimen, the nuclei of nerve-cells are of considerable size. They are globular, vesicular and very *poorly supplied with chromatin*. The most striking feature is a large **nucleolus**, which has taken the methylene blue, i.e., the basic dye; however, it is basophilic only in the restricted sense, such as we found present in the case of *Nissl's* granules. Aside from the nucleolus or nucleoli the nuclei of our specimen are distinguished by a red framework, which is continued into the nuclear membrane, the latter being partly of blue color. A similar state of affairs is found in *Biondi* specimens, where the chromatin of the nerve-cells stains chiefly with acid dyes, viz., it is **oxyphilic**, in contradistinction to the chromatin of all other cell-bodies. A few single granules of basic chromatin may be found among the **oxychromatin** of the nerve-cell nucleus.

Fig. 79.—Nerve-Fibres from the Great Sciatic Nerve of the Frog

300. Osmic acid, acid fuchsin. Frozen section.

The peripheral nerve-fibres, which we know to be processes of the nerve-cell, can be most conveniently studied by examining the great sciatic nerve of the frog. We decapitate a frog, destroy the spinal cord, place the animal on its abdomen and split the skin on the dorsal surface of the thigh longitudinally. The triangular sciatic foramen on the edge of the pelvis will be in plain view, its lower pointed angle being bounded by the heads of the pyriformis internally and the gluteus maximus externally. In its depth the nerve can be located by opening the fascia from the apex of the triangle downward. After separating the muscles (iliofibularis and semimembranosus) we can trace the entire course of the nerve down to the bifurcation into two branches (tibial and peroneal). It is accompanied by the artery of the same name, which is cut and removed. The nerve is severed in its upper part, a small piece is placed on a slide and quickly teased with two needles, the latter being placed closely together in the centre of the piece and drawn outward transversely to the direction of fibres. This method will yield a sufficient number of isolated fibres without inducing great structural changes. The cover-glass is quickly placed and covered with a rim of lac.

The remainder of the nerve is secured with needles below and above and *in situ*. Osmic acid, 2%, is applied drop by drop with a pipette. After ten to fifteen minutes the nerve is superficially fixed; it is now excised and placed in a few cubic centimetres of the same solution for twenty-four hours. After washing for twenty-four hours in running water, the specimen is placed in a 2% acid fuchsin solution for forty-eight hours, rinsed several times in water, and very thin longitudinal sections are made thereof on the freezing microtome, which are mounted in levulose.

*Structure of Medullated
Nerve-Fibres.*

In the fresh, well-preserved specimen the nerve-fibres appear as thin, smooth fibres, having a fluctuating diameter. The strongest fibres of the

sciatic nerve are probably ten times the size of the thinnest. The fibres are surrounded by a highly refractive zone, the **myelin sheath**, which at certain intervals is interrupted, impinging on such places on the calibre of the fibre. The places where the sheath dips in have been called the **constricting bands**, or **rings of Ranvier**. When the fibre loses vitality, rational changes take place, principally affecting the myelin sheath; in our specimen they are rapidly advancing. The smooth outline of the myelin sheath becomes irregular and finally dissolves altogether. If a drop of water be added, lumpy masses of *myelin* will be seen protruding from the myelin sheath.

The finer structural details must be studied on frozen sections. Fig. 79 presents three fibres from the sciatic of about the same calibre. Going from within outward the fibre is first wrapped in a very thin red sheath, the **sheath of Schwann**, or **neurilemma** (*ss*), in which we can recognize a nucleus (*sk*). The nuclei are placed at great intervals, always closely approximated to the *myelin sheath*. The latter is represented by a thick cylindrical sheath, stained black by the osmic acid. We can see the sheath partly plastic (optical longitudinal view), partly in section. Each of the three fibres presents a band of *Ranvier*. Here the myelin sheath ends abruptly in a pointed extremity, another starting on the other side of the ring. By these constrictions, which are found in intervals, varying according to the thickness of the fibres, the entire myelin sheath of a nerve-fibre is divided into numerous chained segments, the **myelin segments**. Each segment forms a long pipe, both ends tapering abruptly, so that entrance and exit are much narrowed. The lowest of the three fibres reproduced in Fig. 79 has been cut in such a manner as to exclude the opening within the constriction, thus giving the appearance as if the two segments ended in solid knobs.

Between two myelin segments a plate of cement substance is inserted, which extends outward to the neurilemma. The **cement plate** can be demonstrated by placing a fresh nerve in a 1% silver nitrate solution overnight, which will render it brown or black. Owing to the fact that the silver solution will penetrate through the two openings into the myelin segments, where it is reduced, a Latin cross will be formed. We will see later that the same effect can be produced by vital methylene blue staining.

Each myelin segment is in turn composed of short pieces, which owe their existence to the **indentations of Schmitt-Lantermann** (*sle*). On the fresh, unchanged fibre they are hard to recognize, but become more distinct as the fibre dies. They cross the myelin sheath obliquely from without inward, so that each segment partly covers the preceding one. Under the action of osmic acid the invaginated ends of the segments produce the effect of circular stripes on the myelin sheath in transmitted light. These numerous small segments composing each myelin sheath have been called **cylindro-conical segments**.

Finally we must mention the last and most important part of the nerve-fibre, the **axis-cylinder** (*a*). In form of a red band it runs through the entire length of the nerve-fibre. It is broadest in the centre of each myelin segment, becoming thinner at the constricted parts. It is probable that *intra*

vitam it fills the entire interior of each myelin segment, the so-called axis space. We can distinctly see that it is made up of numerous closely approximated fibrils. The latter are the **neurofibrils**, which enter the neurite at the cone of origin in the nerve-cell. The neurite is thus identical with the axis-cylinder, which, taking on a myelin sheath, constitutes our central nerve-fibre. After leaving the central organ it becomes invested with the neurilemma, and now we have a peripheral myelinated nerve-fibre. The fibres of the sympathetic nerves, on the other hand, lack the myelin sheath, although they possess a neurilemma; they are **non-medullated**, or **Remak's fibres**, so-called. Aside from the neurofibrils the axis-cylinder contains in all probability an interstitial substance of more liquid consistency, in which the fibrils float, the **achsoplasm**, or **neuroplasm**.

PLATE 32

Figs. 80-82.—Human Blood



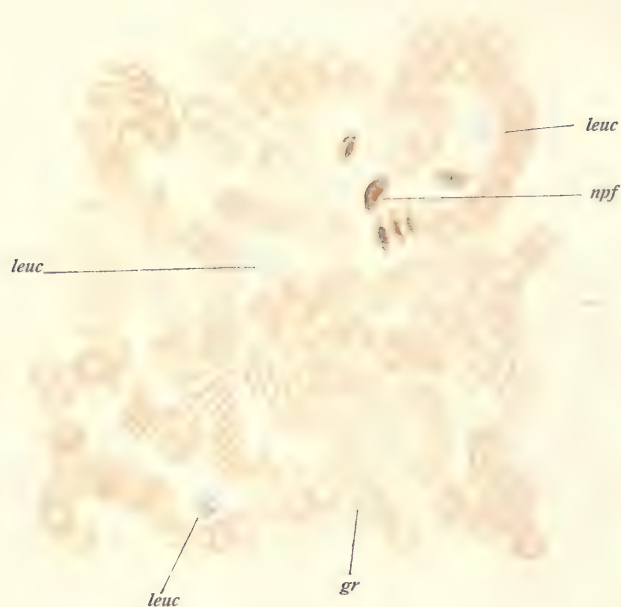


Fig. 80.

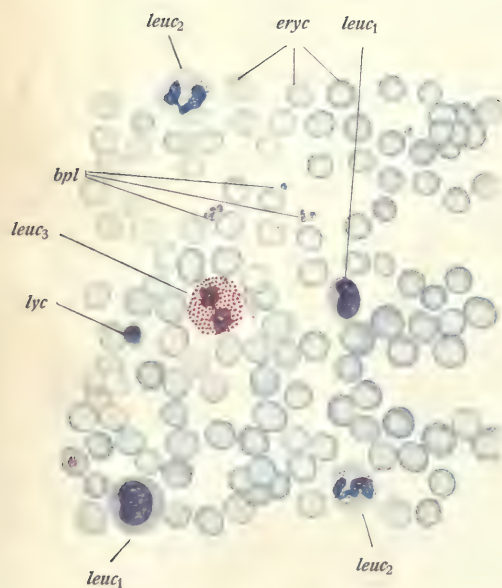


Fig. 81.

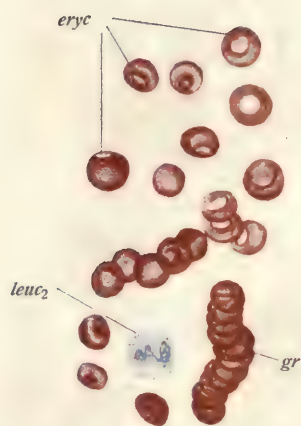


Fig. 82.

7. THE BLOOD

Fig. 80.—Human Blood

600. Fresh, unprepared.

The examination of the elements of the blood in its fresh state belongs to the simplest microtechnical procedures, requiring but rapid execution and painstaking cleanliness of material, viz., slide and cover-glass. To fulfil the latter condition, it is best to use only fresh glasses and to put them in a concentrated solution of sulphuric acid overnight, or at least for a few hours. After the acid has been decanted, the glasses are thoroughly washed in running water until the minutest trace of the acid is removed. Thereafter they are rinsed in distilled water and transferred to a jar containing 95% alcohol. From here they are taken only shortly before use and dried carefully with a clean cloth. The glasses should never be grasped at their surfaces, but only at the edges.

The finger-tip is most convenient for a draught of human blood. Should we desire to examine our own blood, we would select, of course, a finger of the left hand. The finger-tip is thoroughly cleansed with water and soap, rinsed again in water and rubbed dry with a clean cloth. The stab-wound is made with a microscopic lancet, previously sterilized by heat. The flame must not be luminous, as otherwise the specimen will be soiled with soot; a Bunsen-burner is best for the purpose. After the needle has cooled, we make a rapid substantial puncture, compressing the finger slightly on both sides. The first drop is shaken off, the second is caught on the cover-glass, the latter being held with forceps and immediately placed on a slide, specimen side down. After quickly sealing the cover-glass with lac, the specimen is ready for examination.

Red Blood Corpuscles.

Under low power we select a field suitable for our work, i.e., a place where the layer of blood is not too thick, and at once proceed to examine it with the immersion-lens. The entire field appears filled with small round bodies, the **red blood corpuscles** or **erythrocytes**. The attribute "red" seems not justified in our specimen. The color of the corpuscles is a very light yellowish brown; only when a thick layer is present they appear reddish. They rarely lie single, but generally in larger numbers, and they show a desire to form long tortuous chains (*gr*).

Examining a single corpuscle, we find that it is ^{typically} circular, the centre being lighter than the periphery, or *vice versa*. Another corpuscle will appear entirely different: narrow, elongated, biscuit-shaped; between these two extremes all forms of transitional shapes are seen. From these ob-

servations we can conclude that the red blood corpuscles are discs, having a thinner centre and thickened edges, so that our circular form represents the surface view, the biscuit shape the side view. Thus we can explain the difference in light between edge and centre. The centre of the corpuscle acts as a biconcave lens, while the edge has the properties of a biconvex or collecting lens. If we bring the lower surface in focus, the centre will be light and the rim dark; if we focus for the upper surface the reverse will take place, because the focus of the concave lens is situated below, that of the convex lens above the corpuscle, speaking from the point of view of the observer.

In forming chains, the red blood corpuscles lie edge on edge, surface against surface, like the single coins in a stack of money. Hence we speak of a **money-stack formation** or **rouleaux formation** of the red blood corpuscles (*gr*).

Besides the disc-shaped erythrocytes we also find corpuscles which have been so much indented that the disc has given way to a cup. The significance of this phenomenon will be discussed later (*npf*).

White Blood Corpuscles.

Among the masses of erythrocytes we see now and then corpuscles which are essentially different. Their number varies, one generally being counted to several hundred erythrocytes. Their most striking feature is that they do not appear yellow but are colorless, having a faint, bluish lustre. These are the **white blood corpuscles** or **leukocytes**. Our picture shows three such corpuscles (*leuc*). Their size varies; at times they are smaller, other times larger than the erythrocytes. Their shape is also different: they are not disc-shaped, but globular. The most important difference lies in the fact that they have a nucleus. It cannot always be seen in the fresh specimen on account of granules, which often fill the cell-body and may hide the nucleus.

Change in the Blood Corpuscles After the Addition of Acetic Acid.

In order to convince ourselves of the absence of a nucleus in the erythrocytes and the presence of one in the leukocytes, we scratch off the lac on two opposite points of the cover-glass and through one of the openings introduce a small drop of 1% acetic acid, applying a small strip of filter paper to the opposite opening. Examining the specimen now, we find the corpuscles swimming in a strong current; at the same time the erythrocytes lose their color entirely, their coloring matter, the **haemoglobin**, being bucked out by the thin acid; they become fainter and finally disappear almost entirely; we then speak of **blood shadows**. The white blood corpuscles act altogether differently. They do not enter into the stream with the red, but adhere to the slide now and then, perhaps, rolling a short distance. The erythrocytes become more and more indistinct, they are easily recognized, so that we may now form an idea as to their number. The nucleus in each white blood corpuscle becomes more or less prominent, due to the action of the acetic acid; the various shapes of nuclei can, however, be much better recognized in the fixed and stained specimen.

*Effect Produced on the
Blood Corpuscles by
Hypertonic and Hypotonic
Solutions.*

If a 1.5% solution of table salt is used instead of the acetic acid, the erythrocytes will become irregular. The body will show jagged processes. These **mulberry** or **morning star shapes** are caused by shrinking, the latter being due to an exudation of water from the body of the erythrocyte into the hypertonic solution. The opposite, viz., a distention, swelling of the corpuscles can be induced by the addition of a hypotonic solution, e.g., 0.3% saline solution.

Fig. 81.—Human Blood

600. Smear. Osmic acid vapors. Azureosin solution.

The simplest method for the preparation of a good, lasting blood specimen consists in making a thinly spread blood-smear and fixing the same with osmic acid vapors. For this purpose we do not catch the drop of blood with the cover-glass, but with the slide, cleansed in the manner previously mentioned. A clean glass rod is then placed flatly on the slide into the drop of blood and stroking to either side the drop is spread. The slide is now quickly inverted over a flat dish, containing 2–3 cm³ of a 2% solution of osmic acid. The dish should be so small as to be entirely covered by the slide. After half a minute the fixation is completed, and the specimen can be stained in azureosin solution (p. 67). After fifteen minutes have elapsed we rinse in water, dry the specimen and mount it in Canada balsam.

*Varieties of White Blood
Corpuscles.*

The erythrocytes appear in our specimen as pale, bluish green, round or sometimes irregular discs (*eryc*). The leukocytes present different forms. The most common are those which only slightly excel the erythrocytes in size, but are immediately differentiated by the presence of a nucleus, which is characterized by a great variety in shape; it has two or three segments and may appear like a handle, sausage or clover, often being divided into different fragments, which are connected by thin, thready bridges. The cell-body contains pale blue granules. These corpuscles are called the **neutrophilic leukocytes** (*leuc*₂). **Eosinophilic leukocytes** (*leuc*₃) are much rarer in the human blood; their body is characterized by the presence of numerous bright red and quite coarse granules. They are generally slightly larger than the preceding variety, but show no essential difference in the form of their nuclei. The largest cells in human blood are the **large mononuclear leukocytes**. They are relatively rare, two representatives being present in our picture (*leuc*₁). The nucleus is round, oval or slightly indented. The blue cell-body contains no granules. The smallest variety of white blood corpuscles are the **lymphocytes** (*lyc*). They are a trifle larger than the erythrocytes, contain a round nucleus, which almost entirely fills the

cell, and have no granulation. In frequency they range after the neutrophilic leukocytes. Finally we may mention a last variety, which does not appear in our picture and is only rarely found in human blood, the **mast-cells**, which we have already met while studying connective tissue.

Aside from red and white blood corpuscles our specimen presents a third morphological feature of the blood, the **blood-platelets** (*bpl*). They are much smaller than the erythrocytes, occur in various forms and are frequently found in small groups or heaps. In their interior one or more deep blue granules are found, which have also been interpreted as nucleus.

Fig. 82.—Human Blood

600. Smear. Osmic acid. *Biondi* solution.

If the vitality of the corpuscles be destroyed more rapidly than was the case in the last specimen, the shape of the erythrocytes will be altered in a different manner. The procedure is the same as before, excepting that the slide to be used is previously exposed to osmic acid vapors. The blank slide is placed for several minutes over the vessel containing the osmic acid, and the smear is made on the same surface of the slide which had been exposed to the vapors. The smear is again placed over the acid for about twenty seconds. As staining fluid we will not use the azureosin this time, but *Biondi* solution (p. 67) instead.

Cup-Shaped Red Blood Corpuscles.

While the preceding specimen may have shown a few stray cup-shaped corpuscles, the greater number of the red cells have taken on such form in this smear. The same shape is occasionally observed in circulating blood, so that we might consider the cup-shape or bell-shape as the actual vital form of the corpuscle.

The *Biondi* preparation also brings out the various forms of leukocytes very prettily, giving us opportunity to compare and supplement our findings in the preceding specimen.

PLATE 33

**Fig. 83.—Arteries and Capillaries from the Pia Mater of the
Sheep**

Fig. 84.—Capillary from the Pia Mater of the Sheep



Fig. 83.



Fig. 84.

III. THE ORGANS

1. THE CIRCULATORY ORGANS

Fig. 83.—Arteries and Capillaries from the Pia Mater of the Sheep

35. $\frac{2}{3}$. Surface specimen. Silver nitrate.

To study the vascular anastomosis and structure of the vessels we select the pia mater of any of our larger domestic animals. We can obtain and prepare it in the manner described on p. II, 59, and stain it with hæmalum; a better result will be obtained by previously injecting a 1% solution of silver nitrate. We proceed as follows: The head of a slaughtered animal, preferably a sheep, is injected with the solution through the internal carotid artery. For this purpose we locate the artery on both sides, which is not always an easy task, owing to the retraction of the organ. A cannula, armed with rubber tube and pinch cock and filled with the silver solution, is inserted and tied in each of the two vessels. Now we look for the severed ends of both jugular veins and mark them with hæmostats and finally close the vertebral canal by stuffing with cotton tampons. In the manner described on p. II, 47, a funnel is connected by a rubber tube to one cannula, or preferably by a T-shaped glass tube to both cannulæ, and the silver solution injected into the carotids under very light pressure. As soon as the solution returns by the jugulars the latter are closed with the clips and additional 50 cm³ of the solution are injected with very little pressure. In from fifteen to twenty minutes after the completion of the injection the veins and arteries are opened to allow the excess solution to escape. The cranium can now be opened and the specimen prepared in the manner discussed on p. II, 59. It is advisable to wear rubber gloves for this work. After opening the skull and dividing the dura mater the surface of the brain appears colorless, yet slightly milky. Very soon the reduction of the salt will take place and the specimen assumes a brown color. The pieces of pia, mounted on a wax plate, should be left in a large basin of water overnight, then dehydrated, cut into suitable pieces and after applying xylol be mounted in balsam. The nuclei can be previously stained with hæmalum, but little is gained by this procedure and the specimen is apt to lose in transparency.

*The Branching of
the Vessels.*

Our transparent specimen enables us to macroscopically distinguish the larger vessels with their branches, which appear as yellowish brown lines. Fig. 83 shows a piece of a small *artery* under low power. Numerous branches

of varying sizes spring from the main trunk, the branches dividing in turn into smaller ones and ending in very fine tubules, the **capillaries**. The latter anastomose among themselves, forming a network of delicate vessels, the **capillary net**, from which the small *veins* develop. We also learn from our specimen that the larger branches of the arteries anastomose, so that the blood-stream may reach the capillary net in many ways. Such is not the case everywhere, e.g., in the spinal cord the arteries do not anastomose, the capillaries only communicating among themselves. Arteries which do not anastomose are called **endarteries**.

Structure of the Vessel-Wall.

Low power already shows a structural design in the vessel-wall, finer and coarser dark brown lines running either longitudinally or transversely to the direction of the vessel. In the larger branches the transverse lines are most prominent. The smaller the branches are, the more predominates the longitudinal striation. We shall proceed to make a close study of some of the parts of our specimen under higher power.

Fig. 84.—Capillary from the Pia Mater of the Sheep

(Place marked δ in Fig. 83.) 300.

*Structure of the
Capillary Wall.*

First let us select the place marked δ in our picture, which represents a capillary springing from a small arterial branch. The net-shaped structure of the wall strikes our eye immediately, reminding us of Fig. 20, the flat mesenteric epithelium. We have here similar cells, which have been exquisitely outlined by the silver salt having been reduced by the cement substance. The **vascular epithelium** consists of thin flat cells of varying shape, mostly elongated, the two ends being drawn out to points. Their long axis coincides with the longitudinal axis of the vessel; they are connected among themselves by serrated edges. Where several cells come in contact, viz., at the crossing points in the network, the cement substance is present in larger amounts. Such places have received the name of **stomata**. This is where the migrating cells, which we have so often met in the tissues, are supposed to leave their vessel. By changing our focus repeatedly we convince ourselves that this flat epithelium forms the inner lining of the capillary lumen. Ordinarily this epithelial lining of the vascular system is called **endothelium**. Externally to the epithelium the capillary wall is strengthened by a pale yellow layer, the **adventitia capillaris**, in which we cannot detect any structural details. Opinion is divided as to the composition thereof. Some declare it to be composed of connective tissue, others claim it to consist of a network of contractile cells.

PLATE 34

Fig. 85.—Smallest Artery from the Pia Mater of the Sheep

Fig. 86.—Artery from the Pia Mater of the Sheep

Fig. 85.—Smallest Artery from the Pia Mater of the Sheep

(Marked + in Fig. 83.) 300.

Small Arteries.

Selecting the small arterial branch (+), from which the capillary arose, we find that, besides the net-formed epithelial structure, there is a transverse marking present. The epithelial tube is enclosed by rings of smaller or larger calibre. If we focus the wall of the vessel they appear as narrow fibres, their tapering ends joining; if the centre of the vessel is focussed we see these fibres in optical cross-section. They are arranged in a single layer closely under the epithelium, each distinctly separate from the other and almost circular in cross-section. This form, however, does not correspond to conditions during life, when they are closely approximated and of irregular shape. We must therefore consider our specimen shrunken, due to the action of the silver nitrate. The fibres are embedded in a layer similar to the adventitia capillaris, which extends to form the outer covering. The fibres are **smooth muscle fibres**, which are the essential factors in the elasticity of the vessel. We thus have three layers in these small arterial branches, the innermost **tunica intima**, made up of a single layer of flat epithelium; covering this the **tunica media**, consisting essentially of the smooth muscle fibres, and finally the **tunica adventitia**, which forms the outer layer and also serves for connection with neighboring organs.

Fig. 86.—Artery from the Pia Mater of the Sheep

(Marked × in Fig. 83.) 300.

We will finally examine the trunk artery itself and find that the wall shows the same structural conditions as the previous one, but with the increasing calibre of the vessel the wall has become thicker; this increase in thickness has mainly affected the media and adventitia. Instead of a single layer of smooth muscle, the media now contains five or six. Externally to the media we recognize an adventitia of considerable dimension.

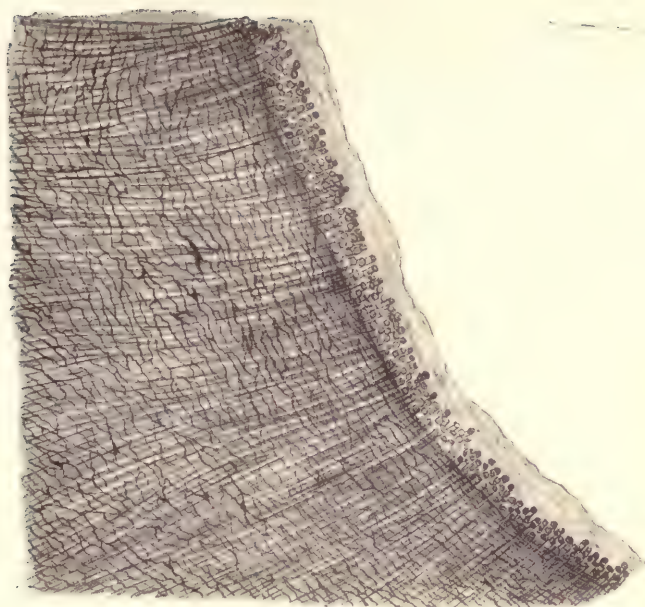


Fig. 86.

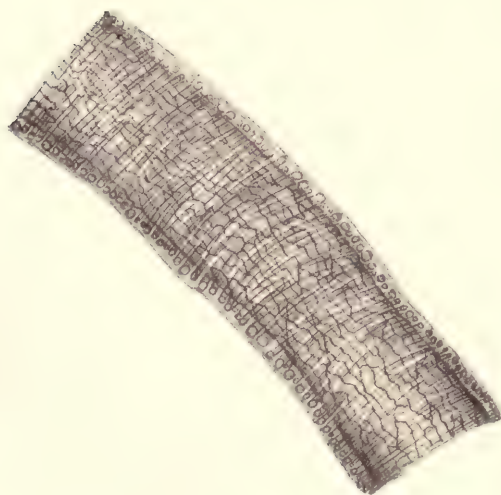


Fig. 85.

PLATE 35

Fig. 87.—Small Blood-Vessels from the Carpus of a Child

**Fig. 88.—Transverse Section through the Human Internal
Carotid Artery**

Fig. 89.—Longitudinal Section through the Human Femoral Vein

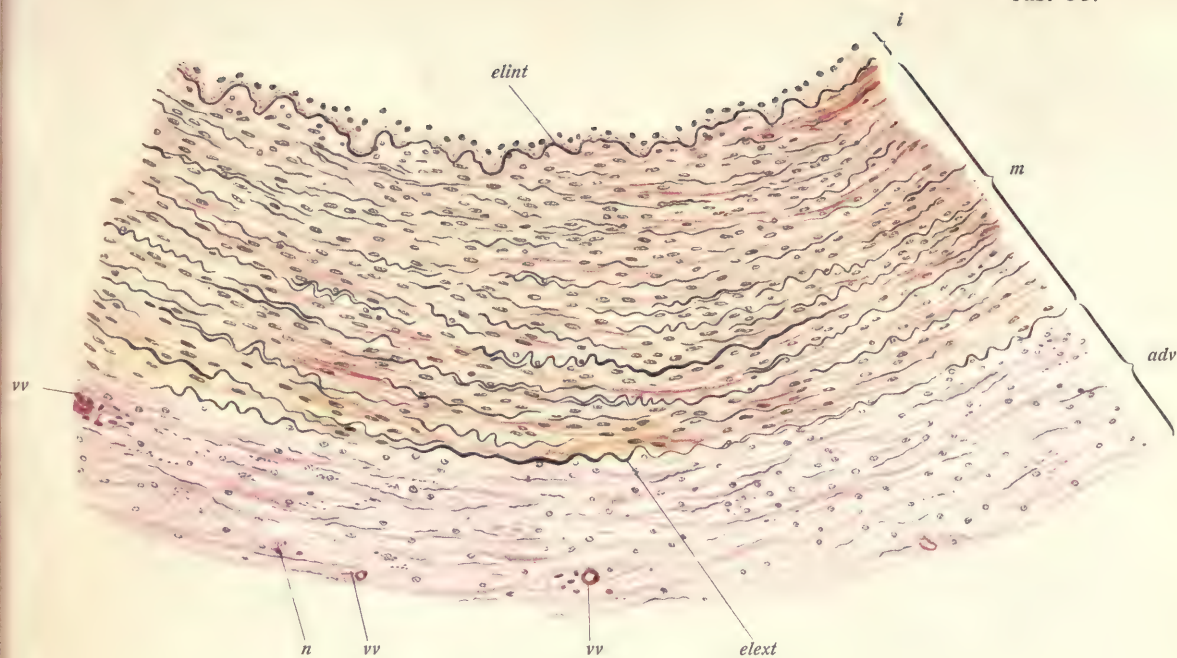


Fig. 88.

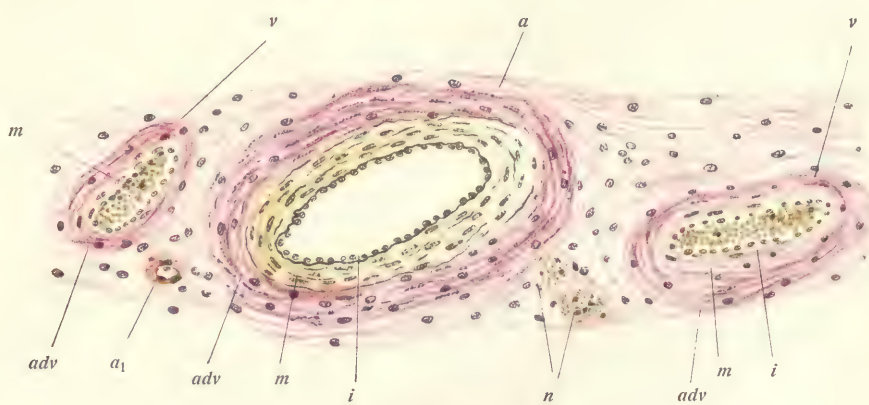


Fig. 87.



Fig. 89.

Fig. 87.—Small Blood-Vessels from the Carpus of a Child

125. Formalin. Frozen section. Iron hæmatoxylin, resorcin fuchsin, picrofuchsin.

A detailed study of the structure of vessels shall be made on a few additional frozen sections. Small arteries and veins can be found in all our sections. Very instructive and otherwise (structure of tendons, cartilage, nerves, etc.) interesting specimens are furnished by sections through the carpus of newborn or very young infants. The carpal bones at that time are still purely cartilaginous, so that sections can be readily made on the freezing microtome, after the hand has been severed at the wrist-joint and fixed in formalin. The sections are stained for fifteen minutes in iron hæmatoxylin (p. 57), rinsed in hydrant water and transferred to 70% alcohol. From the latter they are taken and placed in resorcin fuchsin (p. 63) for twenty minutes, thoroughly rinsed in alcohol 95% until no more of the dye is given off, and thence transferred to picrofuchsin (p. 66). After five to ten minutes they are brought to 70% alcohol, to remove the excess dye, dehydrated and mounted in balsam.

Structure of a Small Artery.

Fig. 87 shows the cross-sections of three larger vessels. In the centre we see an artery, the lumen of which is entirely empty (*a*). We immediately recognize our three coats. The **intima** (*i*) is represented by a pale yellow line, forming the border of the lumen and containing a row of nuclei; of course we have here our endothelium. The intima is bounded by a tortuous line, the **elastica interna**. It has stained black in the resorcin fuchsin, thus distinctly showing its elastic nature. We therefore find our intima consisting of epithelium and elastica, to which may be added a small amount of collagenous fibres, which are not shown here.

Going outward we next find the **media** (*m*). The circular muscle fibres with their elongated black nuclei have stained bright yellow in the picric acid. Between the yellow muscle fibres we distinguish small amounts of red collagenous and black elastic fibres.

There is a distinct line of demarcation between the media and **adventitia** (*adv*). The latter consists essentially of connective tissue; between the fibres we see nuclei of fibroblasts, but also elastic fibres, partly longitudinally, partly in cross-section.

Structure of a Small Vein.

On either side of the artery we see two vessels (*v*), which offer an entirely different aspect. They are **veins**, the lumen of which is filled with blood, this being the rule, as the arteries by the postmortal contraction of

their muscles empty the greater part of the blood into the veins. As compared with the arteries, we find little segregation of the layers in the veins. We have an *intima* (*i*), but it consists exclusively of the epithelium and possesses no *elastica interna*. At the first glance we miss the *media* (*m*) entirely. Only by close observation can we recognize externally to the epithelium a narrow ring of yellow muscle fibre, which represents the entire media. The *adventitia* (*adv*) takes the lion's share of the vessel-wall, being made up almost exclusively of connective tissue, only very few elastic fibres being present in contradistinction to the arterial adventitia.

We should modify our findings by saying that the veins vary in structure quite a good deal in different parts of the body, and that among medium-sized veins we find such as have a better developed media and intima; such is the case in, e.g., the veins of the lower extremities.

Aside from these three larger vessels our cut shows the transverse section of a very small artery (*a*) with distinct muscular layer, and also two small nerves (*n*) in section.

Fig. 88.—Transverse Section through the Human Internal Carotid Artery

125. $\frac{3}{4}$. Formalin. Frozen section. Iron hæmatoxylin, resorcin, fuchsin, picrofuchsin.

Structure of a Large Artery.

As a type of a large artery we select the internal carotid of man. The vessel is fixed in formalin, frozen sections are made and stained in the manner described before.

Corresponding to the increase in calibre of the vessel, the *media* has attained considerable size; likewise has the *intima* become more extensive. In the latter we can distinctly recognize a layer separating the **epithelium** from the *elastica interna*, made up of fine collagenous fibrils, which are cut transversely, hence must be arranged longitudinally.

In the *media* we are above all surprised at the increase in **elastic tissue**; the elastic fibres have not only increased in number but also in size. Longitudinal sections will show that the greater part of our transverse sections are not fibres but lamellæ. Thus we are dealing with concentrically arranged **elastic lamellæ**, though we also find elastic fibres, isolated or arranged in net-form. We may therefore say that the elastic element increases correspondingly with the calibre of the vessel, attaining its greatest development in the aorta, where it exceeds the **muscular layer** by far, replacing the latter entirely at the root of the aorta. Besides elastic and muscular tissues, the media of the large arteries also contains a large amount of **collagenous fibres**.

The media is sharply divided from the *adventitia*. An elastic bounding lamella has formed, the *elastica externa* (*elext*). The adventitia has changed but little. It contains scanty elastic fibres and shows numerous lumina of vessels (*v.v.*). The latter are transverse sections of the **vasa**

vasorum, which supply nourishment to the vascular wall. **Nerves** (*n*), too, are found in the adventitia; they are sympathetic in nature, vasomotor, and supply principally the muscles of the media.

Fig. 89.—Longitudinal Section through the Human Femoral Vein

55. $\frac{3}{4}$. Formalin. Frozen section. Resorcin fuchsin, iron hæmatoxylin, picrofuchsin.

The femoral vein is a good representative of the large veins; a fairly long piece is excised, slit longitudinally, secured on a wax plate with needles and fixed in formalin. The following day pieces of the wall, including a valve, are excised, transferred to 5% formalin and frozen sections made thereof parallel to the longitudinal axis of the blood-vessel. The sections are stained in the same manner as the preceding specimens.

Structure of the Valves of Veins.

We find here a similar state of affairs as we have seen in the small veins; the different coats of the wall merge without any distinct line of demarcation. In contradistinction to the smaller this large vein is exquisitely strong in muscular tissue. With a view to topographical relationship we must needs notice at once the valve, which arises by a broad base from the wall of the vein, projecting in a tapering curve into the lumen of the vessel. The free edge, which would be represented by the apex of the process, is not shown in our picture. The valve describes a curve, the convexity of which is toward the peripheral extremity of the vein, the concavity being toward the heart. The space between the concavity of the valve and the wall of the vein has been called the ***sinus of the valve*** (*klst*).

To study the structure of the wall of the vein, we will begin within and go outward. The ***intima*** (*i*) shows a fairly strongly developed network of elastic fibrils underneath the epithelium at the lower part of our picture, which is continued at the convex surface of the valve, but not at the concave surface, so that we can always easily differentiate which is the peripheral and which the central surface of the valve. On the latter the intima merely consists of epithelium. Only where this surface joins the base of the valve the elastic fibres begin to reappear and gradually increase in number until the next valve is reached.

The ***media*** acts much the same way. It increases centrally toward the valve, reaching the maximum state of development at the base of the valve. It is composed of circular muscle bundles, separated by well-developed collagenous and very scanty elastic fibres. In the veins we never find such an accomplished elastic coat as we have seen in the large arteries. From the base of the valve the muscle bundles can be traced into the valve itself for some distance, but they rapidly grow thinner and scantier and finally disappear altogether, so that the bulk of the valvular wall consists simply of collagenous tissue containing a few elastic fibres. On the other side of the valve

the media becomes considerably thinner and only gradually increases in muscular tissue.

Without any distinct boundary the media merges into the ***adventitia*** (*adv*), the only particular feature of the latter being the presence of irregularly distributed, longitudinal muscular bundles. It is made up largely of collagenous material, but also contains a moderate amount of elastic fibres. In numerous places we can see the vessels (*bg*), which supply the nourishment to the vessel-wall.

PLATE 36

**Fig. 90.—Transverse Section through the Human Vena Cava
Inferior**

**Fig. 91.—Transverse Section through the Wall of the Right
Ventricle of a Child**

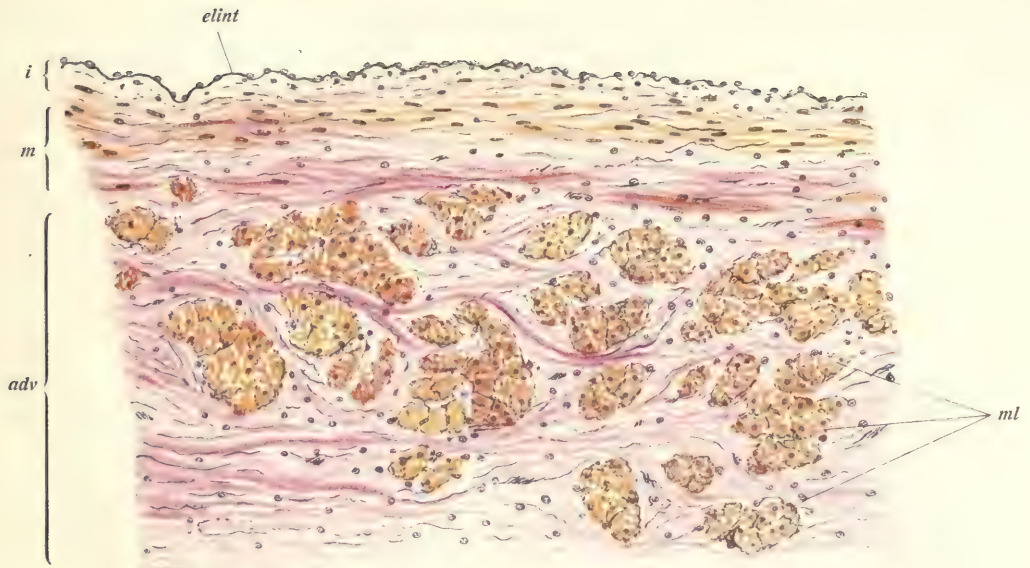


Fig. 90.

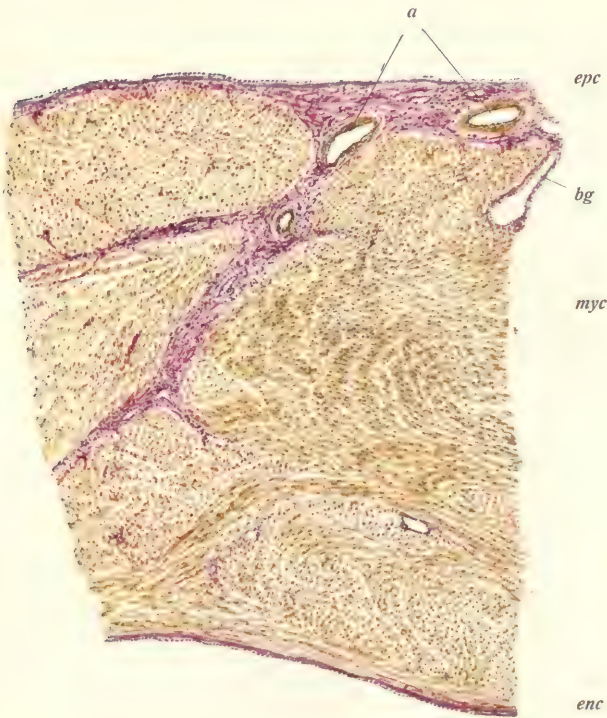


Fig. 91.

Fig. 90.—Transverse Section through the Human Vena Cava Inferior

125. $\frac{3}{4}$. Formalin. Frozen section. Iron hæmatoxylin, resorcin fuchsin, picrofuchsin.

Structure of the Vena Cava.

A last specimen shall give us some information regarding the structure of the vena cava. The preparation does not differ in any respect from that of the preceding specimens.

The **intima** (*i*) is very thin, but has a distinct, although thin and incomplete, elastica interna (*elint*). The **media** (*m*) is well developed, and besides some scanty elastic tissue contains a considerable layer of circular muscle fibres. It merges without any distinct boundary line into the **adventitia** (*adv*), in which we find powerful, transversely cut bundles of smooth muscle (*ml*). The bundles are framed in elastic fibres, running longitudinally and obliquely. Between the muscle bundles strong strands of connective tissue appear, mingled with elastic fibres.

Fig. 91.—Transverse Section through the Wall of the Right Ventricle of a Child

35. $\frac{3}{4}$. Formalin. Frozen section. Iron hæmatoxylin, resorcin fuchsin, picrofuchsin.

We have selected the heart of a child for demonstration of the structure of the heart; it is best to fix it *in toto* by injection. The heart is removed shortly after death, as much of the aorta being included as possible. A wide cannula is introduced into the latter and warm *Ringer's* solution is injected under slight pressure (p. 25). If a specimen has been taken from an animal immediately after death, the fluid, flooding the heart muscle through the coronary vessels, will produce well-regulated contractions. After about 100 cm³ of the fluid have been injected the latter is replaced by a 10% solution of formalin and the hardened organ is now suspended in a large quantity of the solution. After two days small pieces are excised from the different regions, transferred to 5% formalin and sectioned on the freezing microtome. The sections are stained in the same manner as were the arteries and veins.

Structure of the Cardiac Wall.

To gain a more general aspect, we have selected the right ventricle for examination. Externally we find the ventricular wall surrounded by a layer,

consisting mainly of connective tissue, the **epicardium** (*epc*), which lacks development in the infantile heart, but attains a considerable thickness in the adult. The epicardium is separated from the pericardium by a simple layer of flat cells. Beneath this epithelium we come to a stratum of elastic fibres, which are likewise found between the collagenous fibres throughout the epicardium. They are better developed in the adult heart, forming actually elastic coats. In the epicardium we also find the blood-vessels which nourish the heart muscle, together with large amounts of connective tissue, protruding inward. Our picture shows two views of a larger branch of the right coronary artery (*a*) and one of the anterior coronary vein (*v*). The epicardium merges externally into the pericardium, which latter is very similar in structure.

The largest part of the cardiac wall is taken up by the **myocardium**, the heart muscle. The structure of the fibres thereof has been considered previously (p. II, 110). They form smaller or larger bundles. The connective tissue separating them is continuous externally with the epicardium, internally with the endocardium. Elastic fibres are rare in the child, but increase in number with age to an extent that they form quite a prominent feature of the adult myocardium. The special arrangement of the bundles of muscles belongs to the chair of descriptive anatomy; in our specimen we can simply speak of an outer and inner layer, which have both been divided transversely, and a median, which has been cut obliquely or longitudinally.

The **endocardium** (*enc*) finally forms the inner lining of the cardiac wall. Similar to the intima of vessels, it consists of a connective tissue base, which encloses varying amounts of elastic nets and lamellæ. In the infantile heart they are few in number; they increase with years, becoming more pronounced in the auricles than in the ventricles. Smooth muscle is also found in the endocardium to the extent in which we discovered it in the intima. The boundary against the ventricular cavity is formed again by a simple layer of flat cells.

PLATE 37

Fig. 92.—Section through the Base of an Infantile Heart

Fig. 93.—Lymph-Gland of the Monkey

Fig. 92.—Section through the Base of an Infantile Heart

15. $\frac{3}{4}$. Formalin. Frozen section. Iron hæmatoxylin, resorcin fuchsin, picrofuchsin.

Structure of the Aortic Valves.

Sections through the base of the heart are very instructive, demonstrating to us the origin of the vessels from the cardiac wall. Fig. 92 shows the middle portion of such a section, including the origin of the aorta. As we have previously mentioned, the muscular element in the aorta (*ao*) gives way to the powerfully developed elastic tissue, finally disappearing altogether. When entering the heart, this mass of elastic tissue divides into three portions corresponding to the valves of the aorta, forming three pads (*po*), which cover the valvular sinuses externally. Our section runs about midway through the slightly shrunken valves, which latter consist principally of collagenous connective tissue. Here again we find circular and longitudinal elastic fibres, a remarkable similarity in structure to the valves of the veins, inasmuch as these fibres are most numerous on that surface, which mediates the closure of the valve. The surface, bordering on the sinus of the valve, is wanting in elastic fibres. The bundles of connective tissue are largely arranged longitudinally, i.e., from fixed to free edge; there are, however, some few circular bundles present. The names of the three valvular segments are: left (*vss*), right (*vsd*) and posterior (*vsp*) semilunar valves.

On the upper border of our picture, on the right hand, close to the muscle-bundles inserted on that side of the root of the aorta, a part of the origin of the pulmonary artery (*pul*) appears, the left semilunar valve segment being included (*v.s.s.*) in the section. It shows very similar conditions as found in the aorta. Going toward the left we find, embedded in the connective tissue, the latter coated by the epicardium, several transverse and oblique sections of large vessels. They represent the bifurcation of the left coronary artery (*acs*).

Nerve-Cells in the Cardiac Wall.

We can also observe the **trunks** of several small **nerves** and masses of **ganglionic cells** (*ggl*), which under high power prove to be multipolar cells, belonging to the sympathetic system.

Furthermore we notice the **left auricle** (*lvh*), the endocardium of which, as previously mentioned, is characterized by the development of numerous elastic fibrils. Its **myocardium** (*myc*) shows most of the muscle-bundles in transverse section.

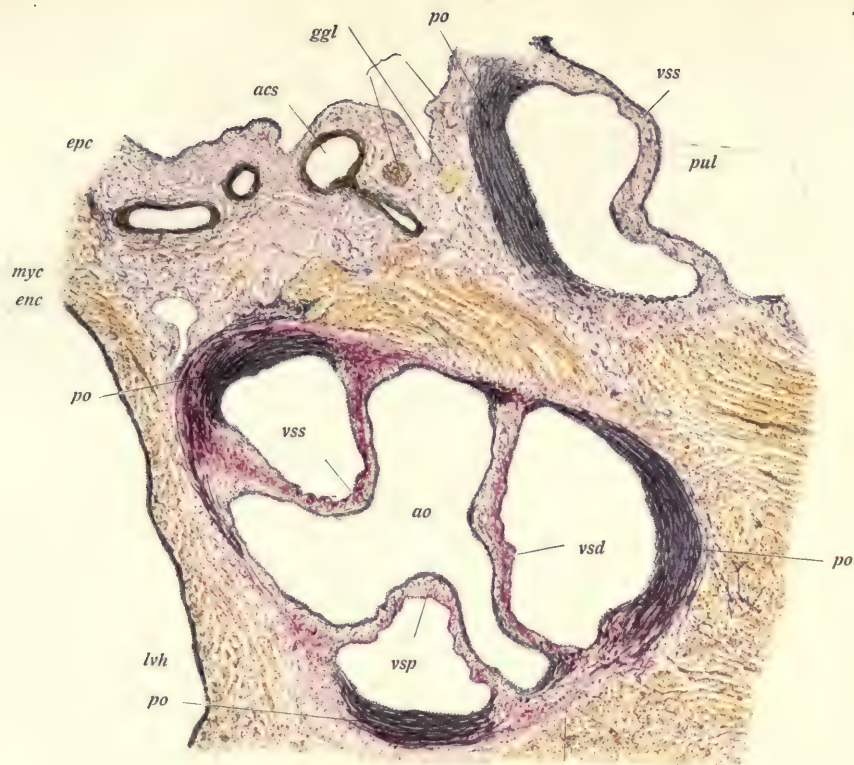


Fig. 92.



Fig. 93.



2. DUCTLESS GLANDS

Fig. 93.—Lymph-Gland of the Monkey

35. $\frac{3}{4}$. Formalin. Frozen section. Paracarmin, phosphomolybdic acid-hæmatoxylin, picric acid.

For the study of the structure of the lymph-node, a lymph-gland of any mammal or of man may be selected. Fixation takes place in 10% formalin. Frozen sections are made and, in order to obtain a precise picture of the collagenous tissue, they are treated as follows: The nuclei are stained with paracarmin (p. 55). Thereafter we wash in 70% alcohol, rinse in water and place the section for ten minutes in a 10% solution of phosphomolybdic acid, wash briefly in water and stain for fifteen minutes in phosphomolybdic acid-hæmatoxylin (p. 57). After rinsing them in water, we place the sections in a concentrated solution of picric acid for two to three minutes, dehydrate in alcohol and mount in balsam. The nuclei will take the red, the collagenous tissue a bluish black, and the erythrocytes the yellow stain.

The Lymph-Gland.

Externally the organ is surrounded by a **capsule** (*k*) containing numerous closely packed collagenous fibres, appearing therefore deep blue-black under low power. Now and then we find within the latter collections of **fat-cells** (*f*) of varying sizes, also slitlike lymph-vessels and blood-vessels (*blg*), which enter the gland at that point. The capsule sends processes, containing collagenous fibres, into the gland, the so-called trabeculæ (*tr*), which ramify in the interior of the organ, forming a network. The amount of collagenous fibres contained in these trabeculæ varies, as our specimens show, being greatest when near a blood-vessel (*tr*). The latter follow the course of the trabecula from the capsule to the medulla of the gland, their adventitia helping to strengthen the collagenous framework of the organ.

The second principal component of the organ is the **parenchyma**; it is composed of **reticular connective tissue**, such as we have met in a previous specimen (p. II, 63), and **lymph-cells**, which are situated in the meshes. We designate such reticulated connective tissue, infiltrated with lymphocytes, as **adenoid tissue**. Corresponding to the trabecular system the parenchyma is divided into an outer superficial layer, the **cortex** (*r*) and a **medulla** (*m*), filling the interior of the gland. Within the cortex the parenchyma forms the **cortical follicles** (*rfo*), pyriform masses, separated from each other by trabeculæ. The rounded base is directed toward the capsule, while interiorly the follicle tapers and forms a strand, which, after entering the medullary substance, becomes a **medullary strand**, joining neighboring strands to form a network.

*Channels of the
Lymph-Stream.*

Cortical follicles and medullary strands do not, however, fill the entire interspaces of the trabecular system, but fissurelike spaces remain between the two. This fact is illustrated very conclusively under the capsule of our specimen. There the surface of the follicle is separated from the capsule by a large space, a **lymph-sinus** (*lys*). These sinuses are crossed by numerous collagenous fibres, connecting the capsule with the follicle. The afferent lymph-vessels, vasa afferentia, lead to the sinuses situated under the capsule. Similar conditions prevail in the medulla; here, too, the medullary strands are separated from the trabeculæ by clefts, lymph-sinuses (*lys*₁), which are in direct communication with the sinuses found under the capsule, so that the lymph is conveyed from the cortex into the medulla, where it streams through a labyrinth of sinuses surrounding the medullary strands; in the so-called hilum it is finally collected by a small lymph-vessel, the vas efferens, whence it leaves the organ.

PLATE 38

Fig. 94.—Lymph-Gland of a Child

Fig. 95.—Spleen of a Child

Fig. 96.—Splenic Sinus from the Human Spleen

Fig. 94.—Lymph-Gland of a Child

550. Formalin. Frozen section. *Biondi* solution.

For the purpose of acquainting ourselves with the specific element of the lymph-gland, the lymphocytes, we stain some frozen sections of a human lymph-gland, which have been fixed in 10% formalin, in *Biondi* solution (p. 67).

Formation of the Lymph Corpuscles.

In the previous specimen we were able to recognize within each cortical follicle a light centre. Searching for such a field in our specimen, we find under high power the red-stained fibres of the reticulum without any difficulty. The cells of the reticular tissue are also very distinct, but in order to demonstrate their anastomosing processes we should have to resort to the gold-method (Fig. 43). Within the meshes of the reticulum we see our lymphocytes corresponding absolutely with those elements in the blood which were introduced as lymphocytes (p. II, 129). They are small cells containing a globular nucleus, rich in chromatin, which is surrounded by a narrow zone of protoplasm. These cells are manufactured in the lymph-glands. They are carried away in the lymph-stream through the lymph-sinuses, and *via* lymph-vessels and thoracic duct gain entrance into the blood circulation. How this constant loss is made up is also illustrated in our specimen. Mitotic propagation of the corpuscles takes place in these **germ centres**; our specimen shows three divisions of nuclei. The blood-vessels, serving to nourish the gland, pierce far into the germ centres, as shown by the capillaries containing blood corpuscles.

Fig. 95.—Spleen of a Child

35. $\frac{3}{4}$. Formalin. Frozen section. *Biondi* solution.

The structure of the human spleen can be demonstrated to best advantage on an infantile specimen, fixed in formalin. Frozen sections, which should not be too thin, are stained in *Biondi* solution.

Spleen.

First of all we select a field closely under the surface which shows under low power that externally the organ is surrounded by a strong **red capsule** (*ka*). In the human specimen it consists mainly of connective tissue with few intermingled smooth **muscle fibres**. The latter are more numerous in

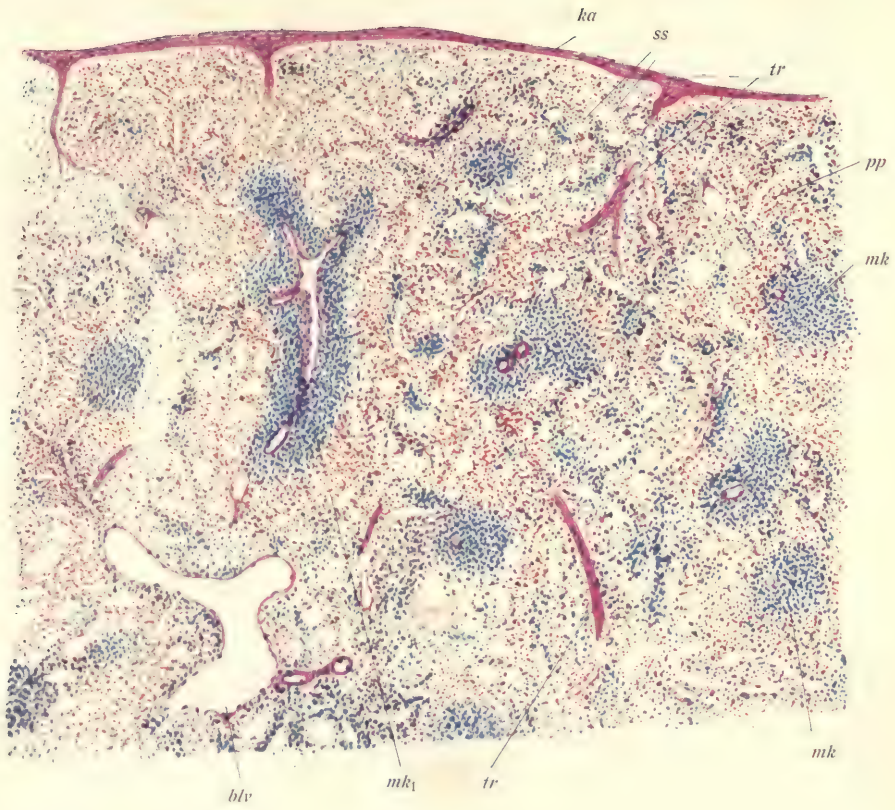


Fig. 95.

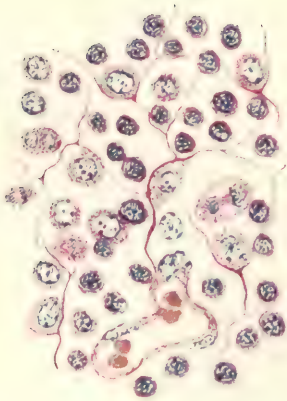


Fig. 94.

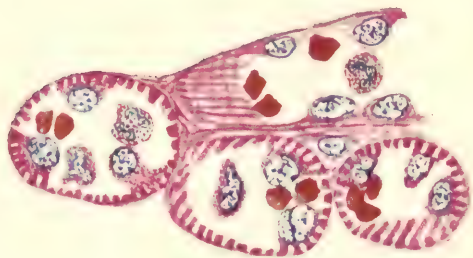


Fig. 96.

animals. Corresponding to the arrangement, found in the lymph-gland, we have **trabeculae** (*tr*), also called **splenic beams**, entering the organ from the capsule. Here they are not so close together, as was the case in the previous specimen. In the interior we recognize them as more or less broad, bright red bands, which can only be traced for a short distance. Again the spaces, included by the trabeculae, similar to the case of the lymph-gland, are filled with pulp (*pp*). The latter has largely taken a deep orange color; since we know that the orange stain in *Biondi* solution is only taken by the *red blood corpuscles*, the latter must needs form an important constituent of splenic pulp. It is a fact that the blood-vessels play the same part in the spleen which the lymph-vessels had in the gland. Let us scour through our specimen thoroughly in this respect.

*Course of the
Blood-Vessels in the Spleen.*

The larger vessels entering the hilum of the spleen are naturally not present in our section; however, we can see numerous smaller branches of the former, either in transverse or in longitudinal section; we can always identify them by the circumstance that they are surrounded by a large amount of lymph corpuscles, the latter being diagnosed by the deep blue nuclei. The arteries are thus surrounded by a sort of **lymphoid sheath**, which, on cross-section, would afford a picture of a rounded corpuscle, containing in its centre the transverse section of an artery (*mk*). These formations, **Mallighian corpuscles** or **bodies**, so-called (*mk*), are present in our specimen in large amounts, their substances merging into the splenic pulp. In one place (*mk*₁) we find such a corpuscle in longitudinal section, showing how the artery bifurcates into three branches.

These branches finally empty their contents into the **splenic sinuses** (*ss*). The latter we find as wide, plexuslike spaces throughout the pulp. From the sinuses the blood is received by the **pulp-veins**, whence it enters the **lobular veins**, large veins, which run along the trabeculae (*blv* shows one of these lobular veins; at its right we see arteries in transverse section in a trabecula); three pulp-veins enter the lobular vein below. The lobular veins join at the hilum of the organ to form the **splenic veins**.

So much we can learn from our specimen; in reality the course of the blood-stream is much more complicated. The fact is that the small arteries do not only lead into the sinuses, but also empty into the pulp meshes, which in turn open into the sinuses, so that the blood may take one of two courses, direct or indirect.

Fig. 96.—Splenic Sinus from the Human Spleen

550. Sublimate. Paraffin embedding. *Biondi* solution.

The finer structural relations of the human spleen can be shown better in the adult specimen. Small pieces are fixed in 3% sublimate solution and embedded in paraffin in the usual manner. Sections, 5–10 μ in thickness, are stained in *Biondi* solution (p. 67).

Structure of a Sinus.

This is extremely interesting. Fig. 96 shows the channels, partly in transverse, partly in longitudinal section, revealing a most peculiar structure in the walls. Externally the sinus is bounded by a structureless **sinus membrane**, which is internally lined with the **sinus epithelium**. The cells consist of bodies and secondly of processes. The body, containing a nucleus, projects far into the lumen of the sinus, sending off its processes to the sinus membrane. The shape of the processes can barely be distinguished in our specimen. On the upper right hand the sinus and the epithelial cells have been cut longitudinally, the processes appearing as long, closely approximated, parallel bands. At the left side a sinus has been cut transversely, the processes appearing as short pegs, connecting the cell-body with the sinus membrane. The two sinuses at the right of the former have been cut obliquely, showing the processes, partly in transverse, partly in oblique section. We may thus summarize our findings by saying that the processes are long ledgelike projections of the cell-body, which are implanted on the sinus membrane parallel to the longitudinal axis of the sinus. These **rod cells**, so-called, probably are of contractile nature. A second system of fibres may be observed in the sinus, cut longitudinally; they are arranged circularly around the sinus, being inserted between the rod cells and the sinus membrane.

Red and white blood corpuscles are seen in the lumen of the sinus; the former are probably destroyed in the spleen, while the latter, as was the case in the lymph-gland, are manufactured here also. They are abundantly found in the pulp, whence they enter the splenic sinus either directly or by actually piercing the sinus membrane.

PLATE 39

**Fig. 97.—Malpighian Corpuscle; Longitudinal Section from the
Human Spleen**

Fig. 98.—Thymus of the Newborn



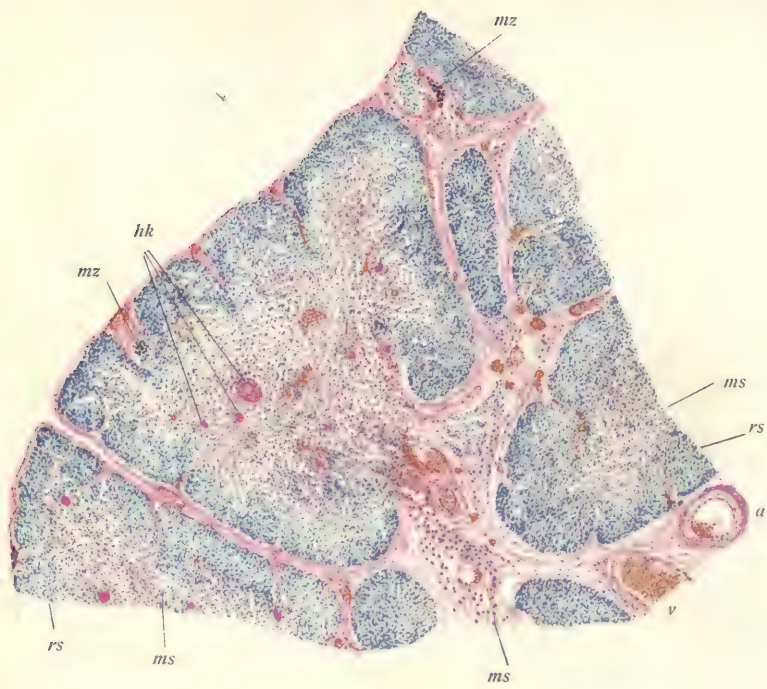


Fig. 98.

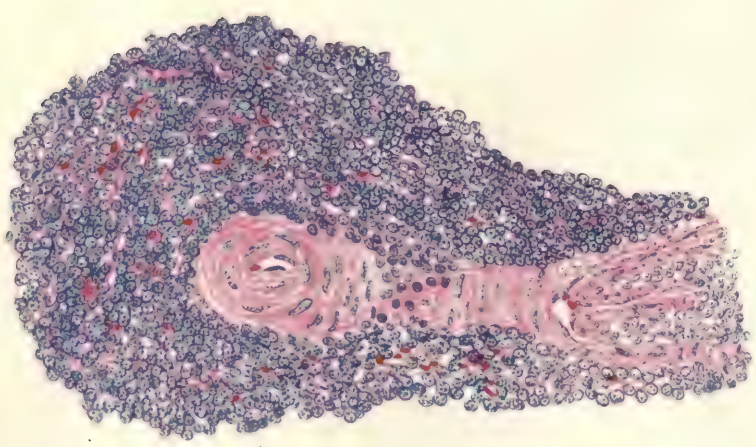


Fig. 97.

Fig. 97.—Malpighian Corpuscle; Longitudinal Section from the Human Spleen

300. $\frac{3}{4}$. Sublimate. Paraffin embedding. *Biondi* solution.

*Malpighian Bodies
in the Spleen.*

Fig. 97 shows a Malpighian corpuscle from the same specimen from which Fig. 96 was taken. Running along the axis of the corpuscles we see the artery with its strongly developed muscular coat. On the right hand it abruptly divides into numerous branches, forming a so-called **penicillus**. After a lengthy course the branches empty into the splenic sinuses.

The corpuscle itself is formed of reticular tissue, the meshes of which are filled with lymphocytes. The reticulum is continuous with that of the surrounding pulp, hence we always expect to find erythrocytes among the lymphocytes. After the artery has divided into the penicillus, the surrounding lymphoid tissue becomes lost, and the vessel is later on wrapped in the so-called **capillary capsule**.

2. DUCTLESS GLANDS

Fig. 98.—The Thymus of the Newborn

35. $\frac{3}{4}$. Formalin. Frozen section. *Biondi* solution.

If we desire to study the thymus at its height of development, we must examine it during the first year of life. Ten per cent. formalin serves for fixation. The frozen sections are stained in *Biondi* solution (p. 67).

The Thymus.

The section shown in Fig. 98 shows under low power a distinct **lobulate** arrangement. The connective tissue capsule, surrounding the entire organ, pierces between the various lobules, dividing the gland into macroscopic lobes, which in turn consist of smaller lobules. On the surface other smaller lobules are formed by the capsule, reminding one of the cortical follicles in a lymph-gland.

Each lobule is filled with the basic substance of the entire organ, the **medullary substance** (*ms*), containing numerous vessels (*a*, *v*). Thus we have the formation of a central **medullary substance**, which is rich in large blood-vessels and has a more reddish color in our specimen, and a surrounding blue **cortical substance** (*rs*), which is rich in small vessels. When unstained, the former will appear light, the latter dark.

The frame of the entire lobule of the thymus is made of **reticular connective tissue**, the meshes of the cortical substance being packed full of **lymphocytes; nucleated red blood corpuscles** are also encountered. In this respect the thymus therefore resembles a lymph-gland. The medullary portion also shows a similar reticulum, the difference being that lymphocytes are very scarce within its meshes. On many places we furthermore notice cells, arranged in strands and strata, which cannot deny their epithelial origin and which impart the reddish color to the medullary substance.

The main characteristics of the medullary substance, however, are the **Hassall corpuscles**, so-called (*hk*), which may attain such a considerable size as to be recognized by the unaided eye. In our specimen they distinguish themselves by their intensely red color; they are only found in the medullary substance, never in the cortex.

PLATE 40

**Fig. 99.—Hassall's Corpuscle from the Thymus Gland of the
Newborn**

Fig. 100.—Thyroid Gland of Man

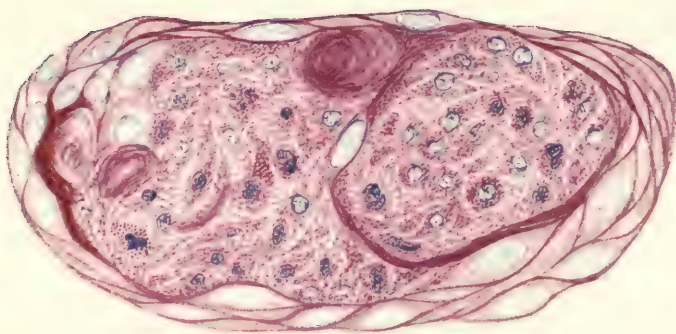


Fig. 99.

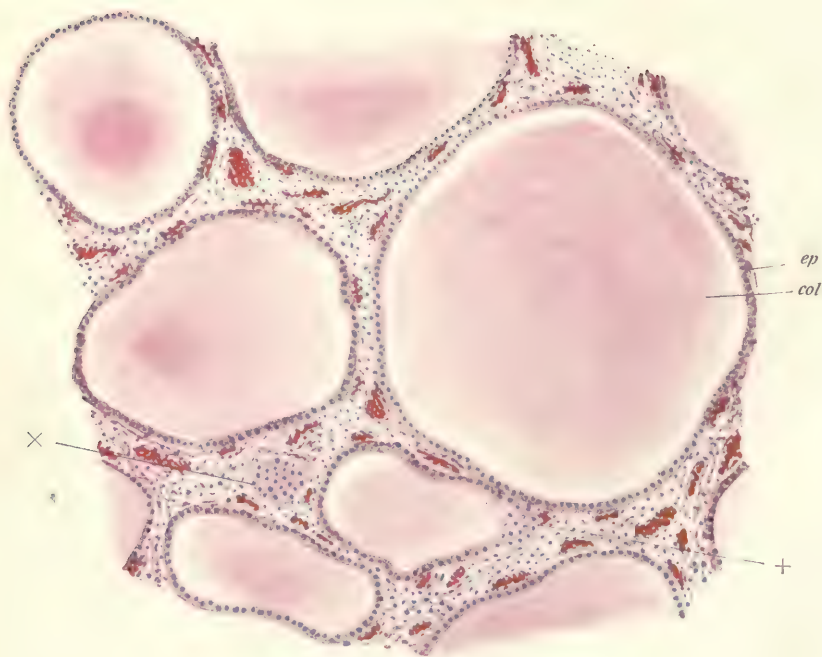


Fig. 100.

Fig. 99.—Hassall's Corpuscle from the Thymus Gland of the Newborn

550. Formalin. Frozen section. *Biondi* solution.

Hassall's Corpuscles.

Looking at a large corpuscle with high power, we will find the following picture. Externally the corpuscle is enveloped by elongated, dishlike cells. The latter are either non-nucleated or possess an extremely large, pale, swollen nucleus. The interior of the corpuscle is filled with a granular mass, studded with nuclei. We must consider the latter partly as leucocytes, partly as epithelial cells undergoing retrogressive changes. We also find lumpy masses, reminding one of starch globules, which have also been formed by conglomeration and degeneration of epithelial cells.

As shown in our specimen, the simple corpuscles may unite and take on new layers to form the large macroscopic formations.

Fig. 100.—Thyroid Gland of Man

175. Formalin. Frozen section. *Biondi* solution.

The freezing method is ideal for the preparation of the thyroid gland, since it is the only method which does not shrink the colloid material, the secretion of the thyroid. Small pieces of the organ are fixed for twenty-four hours in 10% formalin, followed by 5% formalin. Frozen sections are stained in *Biondi* solution (p. 67) and mounted in balsam.

Thyroid Follicles.

The thyroid gland shows very simple structure. Low power demonstrates the **lobulate structure** of the organ. The various lobules are separated by connective tissue, being composed in turn of numerous **thyroid follicles**. These are round or elongated, closed spaces, lined by epithelium and filled with secretion. In our specimen the follicles are variously sized and cut in different planes. Not infrequently we find between the follicles smaller or larger cell complexes, which do not show the typical arrangement (X). These are such places where only the follicle wall, not the lumen, has been cut, i.e., flat surface sections.

The **epithelium** (*ep*), lining the follicles, is simple cuboid of varying height. At times the cubes are high, at others they are quite flat. These differences may occur in the same follicle. An increase in the secretion of a follicle increases naturally the pressure within, which acts on the epithelium, making the cells flatter. The cells themselves show nothing noteworthy. The

globular nucleus is surrounded by more or less granular protoplasm. The cells take the red stain with different intensity, some staining pale pink, others deep red. The former have been named **chief cells** by some, the latter **colloid cells**. They must not be regarded as two different kinds of cells with different functions; the colloid cells are merely an evolution of the chief cells by virtue of the colloid material which they have elaborated in their interior, and later they are in all probability cast into the lumen. A factor speaking for this theory lies in the fact that remains of nuclei are not infrequently found in the secretion. There are numerous places where the epithelium does not form a simple layer, but is clumped together (+). These are simply surface sections of the follicular wall, often due to small sacculations of the follicle.

Externally the epithelium is bounded by a structureless membrana propria, which cannot be completely traced.

The lumen of the follicle is filled with a homogeneous mass, the **colloid material** (*col*). It fills the lumen entirely and is in close contact with the epithelium throughout. When specimens are embedded in paraffin, the colloid material will be found more or less shrunken, thereby retracting from the epithelium. The colloidal substance stains red in our *Biondi* solutions, viz., selects the acid dye. It appears homogeneous most often, rarely somewhat granular. As already mentioned, we can detect here and there whole nuclei or the remains of such. On this basis we can form a conclusion as to the manner of secretion of the colloid substance. Entire epithelial cells are cast into the lumen and transformed to secretion. We may assume that in addition to the former process the secretion is also simply emptied into the lumen by the epithelial cells.

Drainage of the Secretion of the Thyroid.

Between the follicles we find loose connective tissue, which contains innumerable **blood-vessels**. In order to have a good representation of the wealth of blood-vessels in this gland, the afferent and efferent vessels should be tied on a living animal, e.g., a cat, and the organ then fixed *in toto*. Our specimen, although the vessels with their orange-red contents can plainly be seen, only gives a mild conception of the abundance of vessels. Worthy of note is the arrangement of the blood capillaries, which are closely approximated to the epithelium, at times even pushing the epithelial cells into the lumen. Besides the net of blood capillaries the follicles are also richly supplied with **lymph-vessels**. They can be demonstrated excellently by introducing the cannula of a Pravaz syringe¹ filled with Berlin blue (p. 74) under the capsule of an organ, excised *in toto*, using very light pressure during the injection. The lymph-spaces are of special importance here, because they receive the colloid material from the follicles, the latter probably rupturing after the pressure within has reached its maximum.

¹ Hypodermic syringe.

PLATE 41

Fig. 101.—Suprarenal Body of a Child

Fig. 102.—Human Hypophysis

Fig. 101.—Suprarenal Body of a Child

100. *Mueller's* fluid-formalin. Frozen section. Hæmalum. Sudan.

When examining the suprarenal capsule, we must primarily seek to obtain an immaculate preservation of the cells; secondly, a preservation as well as color demonstration of its characteristic components, the lipid and the phæochromic substances. In order to preserve and illustrate the phæochromic substance, we must treat the suprarenal body with chrome salts, which give a characteristic reaction with this substance. We chose *Mueller's* solution (p. 31), adding 10% formalin for better cell preservation. The organ is fixed in this mixture for two to three days, the liquid being changed repeatedly. After washing in running water for twenty-four hours we transfer to 5% formalin. Should we now embed in paraffin, the second of the components, the lipid material, would be extracted; we therefore make frozen sections and stain them first in hæmalum (p. 56) for ten to fifteen minutes, wash in hydrant water for an equal length of time, transfer for a few minutes to 50% alcohol, and thence counterstain in sudan (p. 66) for fifteen minutes. After washing the sections briefly in water we mount them in levulose. The suprarenal of newborn, or very recently born children, is to be preferred; the material used must be fresh. Before fixation the organ should be bisected, to avoid crushing the material.

The Suprarenal Body.

The unaided eye may discern the two characteristic features of the organ, the dark brown **medullary substance**, derived, as we know, from the sympathetic nervous system, surrounded on all sides by the light bluish red **cortical substance**.

The Capsule.

Low power shows the organ enclosed in a thick, nucleated connective tissue capsule (*ka*). It contains numerous blood-vessels (*bg*), and small nerve-trunks (*n*), which, piercing the capsule, gain entrance to the cortex; along their intracapsular course we find, more numerous in the animal than in man, collections of sympathetic nerve-cells. Connective tissue trabeculæ enter the cortex from the capsule, forming a system of radiating, communicating tubes, which contain the cortical parenchyma.

The Cortex.

Our picture shows very clearly how the cortical substance forms beams, which, of course, are also radiating and anastomose with their neighboring

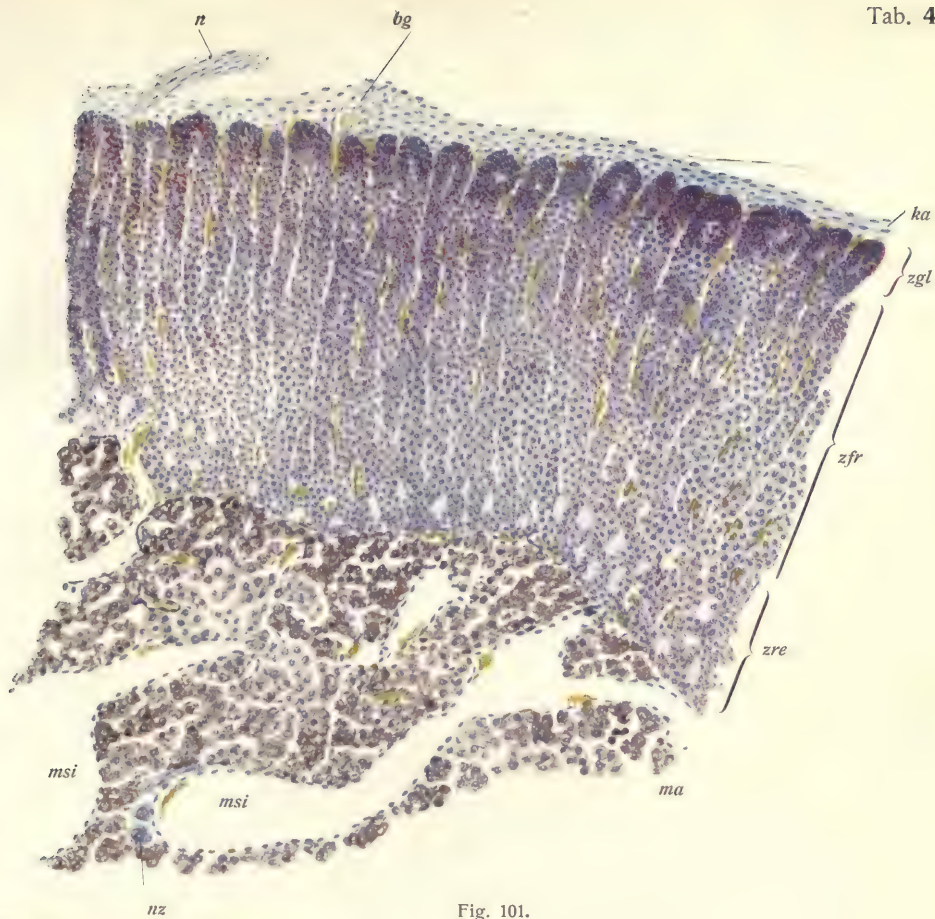


Fig. 101.

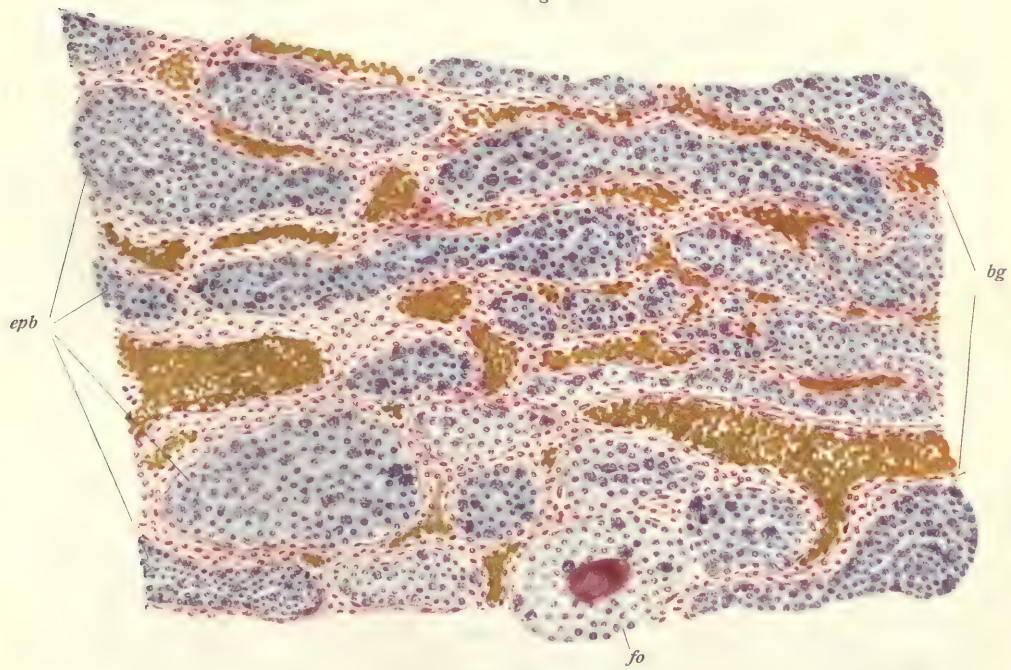


Fig. 102.

fellows. Based on this arrangement, the suprarenal can be divided into three different zones. Closely under the capsule the beams arise in form of loops; they traverse the greater part of the cortex in a straight course and, shortly before reaching the medullary substance, mingle to form a network of round meshes; thus we have, going from without inward: the narrow **zona glomerulosa** (*zgl*); then, occupying the greatest part of the cortex, the **zona fasciculata** (*zfr*), and finally, bordering on the medullary substance, the **zona reticularis** (*zre*). We shall study these three zones under high power.

In the *zona glomerulosa* the different glomeruli, i.e., the loop-formed, communicating, peripheral ends of two neighboring parenchyma tracts, are separated by septa, which here are still fairly strong. We have here elongated narrow cells, arranged transversely to the axis of the tracts. The cells are placed very closely together, the minute cell-body containing small red granules, the **lipoid granules**. In the *zona fasciculata* the cells become considerably larger, their bodies being filled to a greater or lesser extent with lipoid granules. If the specimen has been embedded in paraffin, these granules will be dissolved, vacuoles appearing in their place. Similar conditions prevail in the *zona reticularis*. Here, too, the cells contain lipoid granules, but in addition thereto show, in the adult and in some mammals, a brown pigment.

Medullary Substance.

The medullary portion (*ma*) is sharply defined from the cortical substance, as our picture shows. This is not the case all over, for on some places they merge without any distinct boundary line or may even grow into each other. Here again we find cellular tracts or beams, which appear to communicate in the form of a net; the meshes, however, are considerably larger than in the cortex, being occupied by wide venous blood-spaces, the **medullary sinuses** (*msi*). The cells of the medulla are much larger than those of the cortex. Several rows are present in each tract; in our specimen they appear slightly shrunken, giving the tracts a loose appearance. They are polyhedral cells. The round nucleus lies within a body, which appears dark brown, due to a mass of granules. These are the **phaeochromic granules**. In the fresh, unstained specimen they are pale, colorless granules; their characteristic peculiarity lies in the fact that, when treated with chrome salts, they assume this dark brown color. It is probable that they represent the active principle of the suprarenal body, the **adrenalin**, which by virtue of the thin walls of these wide medullary sinuses can easily gain entrance to the vascular system. Aside from these medullary cells we also find true nerve-cells (*nz*). Our specimen only shows two; other places will show them in greater number. They are sympathetic, viz., multipolar cells, identical with those observed in the cortical substance. Afferent and efferent non-medullated nerves are likewise seen in many places. They form wide plexuses in the medullary as well as cortical substance.

Fig. 102.—Human Hypophysis

200. Formalin. Frozen section. Cresyl violet.

Here again it is of importance to use only fresh material, since the characteristic components of the parenchymatous cells of the pituitary body decompose shortly after death. The organ of a grown person is split with the razor by a median longitudinal section into a right and left half and fixed in formalin 10%. Thin frozen sections are stained in cresyl violet (p. 62).

The organ consists, as we know, of two genetically very different parts. The posterior lobe is derived from the central nervous system. Retrogressive changes, transfiguring it to a mixture of connective tissue and gliomatous elements, cause it to be of little importance to the adult. On the other hand the anterior lobe, owing its origin to the epithelium of the oral cleft, becomes an object of special interest, for recent researches have shown that it furnishes a substance which is very essential to the human economy.

The Anterior Lobe of the Hypophysis.

Even under low power we can notice a certain conformity in substance of the anterior hypophyseal lobe with the suprarenal body and the thyroid gland, viz., the extraordinary wealth in **blood-vessels** (*bg*). Numerous vessels, partly wide and thin walled, form an extensive vascular net, in the meshes of which we find, aside from a rich supply of connective tissue, the parenchyma.

These parenchyma consist of finer or coarser strands or beams, which in turn form a meshwork. The beams (*epb*) are solid, viz., have no lumen, being composed of polyhedral cells, which, according to the thickness of the beam, are arranged in two, three or more rows. The cells are of different sizes, have a globular nucleus, and in regard to their bodies show some important differences. In a large part of the cells we find a pale blue protoplasm, appearing homogeneous or at least very indistinctly granular. Others, however, contain numerous reddish violet granules. Transitional forms between these two varieties of cells can be found in large amount under high power. Evidently these granules are elaborated by the cells and probably represent the secretion of the hypophysis or part thereof, perhaps a pre-stage of it. In the manner observed in the suprarenal body, the secretion is poured into the blood-vessels and by the latter conveyed to the organism.

A second constant component part of the hypophyseal parenchyma is formed by the **follicles**, i.e., closed spaces, lined by epithelium (*fo*). They remind us of the thyroid follicles; their lumen is filled with a reddish violet, homogeneous mass, which corresponds in all microchemical reactions with the colloid substance of the thyroid gland. They may be differentiated from the thyroid follicles by the fact that their epithelial coating is generally not simple but stratified. The epithelia here do not differ in any respect from those of the epithelial beams. In the anterior portion of the anterior lobe follicles are scarce, becoming more numerous in the posterior part.

PLATE 42

**Fig. 103.—Longitudinal Filed and Polished Section through a
Human Incisor**

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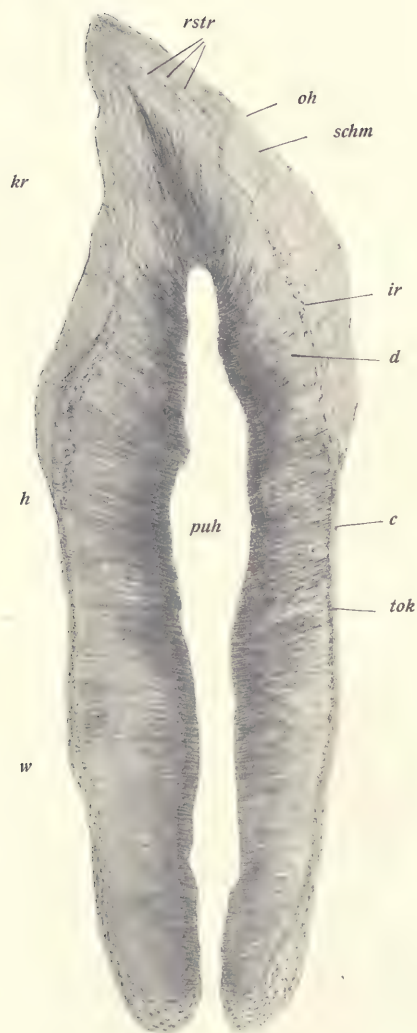


Fig. 103.

3. THE ORGANS OF DIGESTION

Fig. 103.—Longitudinal Filed and Polished Section through a Human Incisor

10. $\frac{3}{4}$.

The technique of preparing teeth is much the same as that used for bone. Thus we may either prepare thin transparent specimens by first macerating the tooth, i.e., extracting the soft parts, and then filing and polishing the same, or we may fix the fresh tooth, decalcify and section it. Both methods have their own advantages and are supplementary. Let us first consider the hard preparation.

Method of Preparing a Veneer of Tooth.

It is quite a difficult task to prepare a good veneer of the entire length of the tooth. A well-macerated incisor is chosen and mounted in a corresponding groove of a wooden block with cover-glass cement (p. 77) in such a manner that one-half of the tooth is free while the other half is buried in the wood. Since the best of files will soon be rendered useless by the glass-like consistency of the adamantine substance¹ of the tooth, a small rectangular hone, such as is used for the sharpening of scalpels, will be found more serviceable. With it we grind down the tooth parallel to the median plane, moistening sufficiently with water, until the pulp cavity has been opened in its entire length. The tooth is now released from its bed, dried, and the ground surface cemented on to a smooth part of the wood. Now we proceed to file down the other side until a thickness of 0.5–1 mm is attained. The work is completed with the file on the slide in just the same manner as bone. We must always be absolutely sure that the section is thoroughly secured by the cement throughout its entire length, as otherwise chips of adamantine substance¹ (p. II, 82) may break off. We can, of course, fill the cavities of the tooth with fuchsin (p. 66), as was done in the bone.

Gross Structure of the Tooth.

Macroscopically our veneer shows the three anatomical parts of the tooth: crown, neck and root. The **crown**, i.e., the free portion of the tooth, is coated with **enamel** (*schm*), which decreases in thickness from above downward. It has a double striation, one fine and radiating, another nearly parallel to the surface, but only in the upper parts of the crown; below they

¹ Enamel.

run at an acute angle to the surface of the enamel. They are the **striae** or **Retzius** in the enamel.

The **root** is that part which is inserted in the bony alveolus; it is coated with the **cement** (*c*), which, contrary to the enamel, increases in thickness from above downward. The **neck** is a portion about 2 mm in length, still covered by cement, which is not buried in the alveolus, but neither is free in the oral cavity, being covered by the **gums**. In the region of the neck enamel and cement come together in a manner so that usually the former is slightly overlapped by the latter. In the cement we can, even under low power, recognize small, stellate cavities, similar to the canaliculi in bone, the **cement cavities**. They are wanting in the neck, appearing only in the upper or middle portions of the root.

The bulk of the tooth consists of the **dentine**. It encloses the **pulp cavity** and lines the root canal, which arises from the former at the highest portion of the root, but it never reaches the surface of the tooth anywhere. The dentine has a radiating striation, due to the dental canaliculi, which traverse it through its entire thickness, opening internally into the pulp cavity. In the coronal portion of the dentine we also notice a simple or stratified layer of jagged air-spaces, running parallel to the boundary between enamel and dentine, the **interglobular spaces**. They disappear in the region of the neck, their place being taken by a stratum of minute vacuoles, situated immediately beneath the boundary between cement and dentine, the **granular layer of Tomes**.¹

¹ English dentist, 1836-95.

PLATE 43

Fig. 104.—Longitudinal Filed Section through the Crown of a Human Incisor

Fig. 105.—Longitudinal Veneer Section through the Root of a Human Incisor

Fig. 106.—Enamel Prisms from a Human Wisdom Tooth

Fig. 104.—Longitudinal Filed Section through the Crown of a Human Incisor. 175.

Enamel.

We will now examine some special fields of our section under high power, selecting first the enamel (*schm*). In it we recognize an irregular, undulating striation, radiating from the dentine border, which is due to its composition of long prismatic fibres, the **enamel prisms**. They wind their way in screw-form from the dentine borderline to the surface, hence appear both in oblique and transverse section. They consist almost exclusively of an organic material. Between the prisms we find intercellular **cement substance**, which is very scanty in the adult tooth. The **striae of Retzius** (*rstr*) appear running obliquely within the enamel. Externally the enamel is surrounded by the likewise calcified **enamel membrane**.

Dentine.

The **dentine** (*d*) making up the bulk of the tooth owes its characteristic appearance to the **dental canaliculi** or **dentine tubules**. In the crown their course is fairly straight, showing, perhaps, slight curving. They taper from the pulp-cavity toward the enamel border. When reaching the latter they branch, anastomose and end blindly, one of the branches not infrequently trespassing on the border-line. In such a case the blind end, often somewhat thickened, lies between the enamel prisms. At times the canaliculi will arch over, forming an arcadelike arrangement. Furthermore, the tubules give off side branches throughout their entire course, their number increasing toward the periphery. The branches anastomose among themselves, but also travel for quite a distance independently, communicating with remote canaliculi.

Our specimen also shows an **interglobular space** (*ir*). These spaces are uncalcified places in the basic substance, into which the calcified substance sends rounded processes, **dentine globules**, so-called. The dentine tubules traverse the interglobular spaces uninterrupted.

About the structure of the basic substance of dentine we cannot gain much knowledge from our specimen. It consists of calcified collagenous fibrils, deposited in strata in such a manner that the different layers run parallel to the surface of the tooth, the fibrils being arranged longitudinally within them, thus crossing the dentine tubules at about right angles. Similar to the case of bone, the **basic substance** forms sheaths around the canaliculi, the **dentine sheaths**, or **Neumann's sheaths**. The latter can be demonstrated by the gilding process, performed in the manner described under bone (p. II, 83).

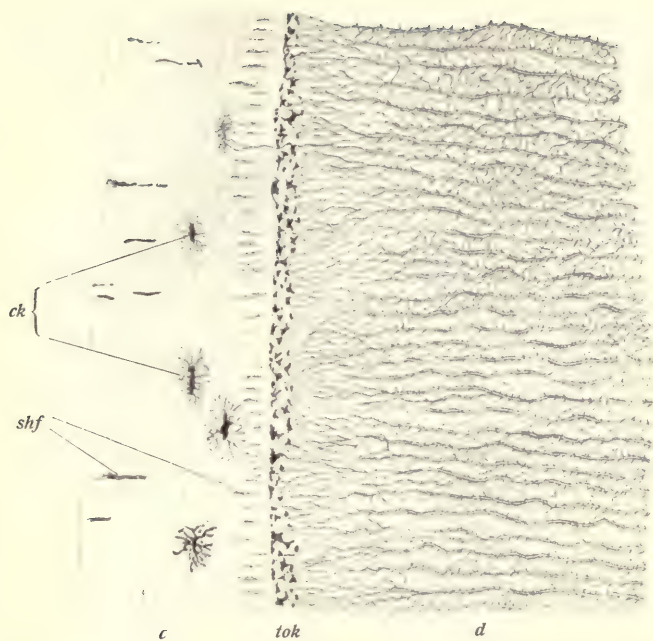


Fig. 105.



Fig. 106.

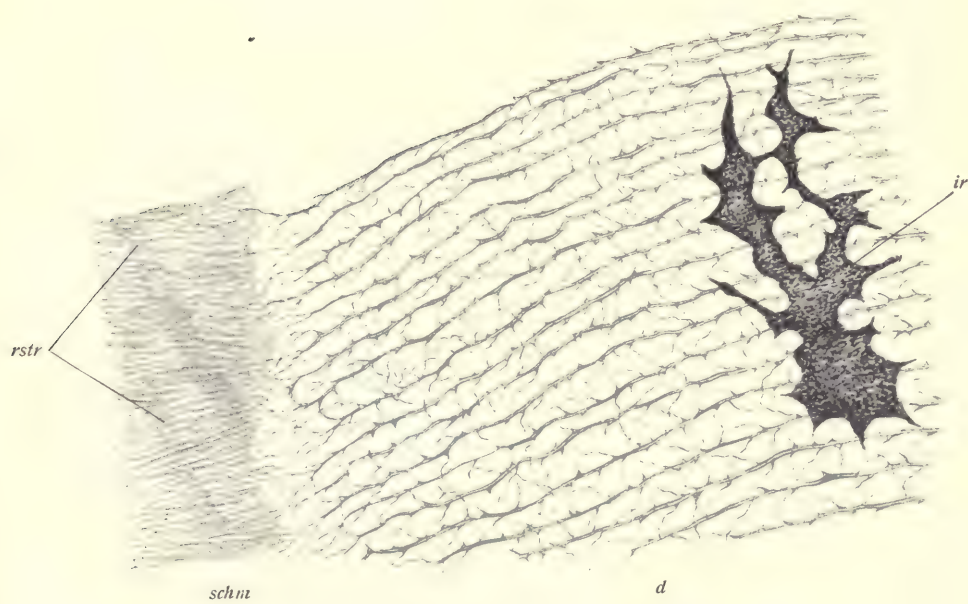


Fig. 104.

Fig. 105.—Longitudinal Veneer Section through the Root of a Human Incisor. 175.

Cement.

An entirely different view presents itself in a filed section through the root of the tooth. Instead of enamel we find the outer covering to consist of **cement** (*c*). This really is genuine bone substance, which, at least in the thicker layer of cement, shows distinct lamellæ. Canaliculi (*ck*), too, are found with their typical processes, although few in number; they are wanting at the neck of the tooth, but increase in number toward the root.

A striking feature is presented by canaliculi, which are situated vertically to the surface of the cement (*shf*). In our specimen we see a continuous layer of them at the cement-dentine border, but also find them sporadically in the peripheral layer. They are **Sharpey's¹ fibres**, bundles of collagenous fibres, traversing the cement lamellæ transversely. Since they are not calcified, they appear in our macerated specimen as hollow tubes in the calcified basic substance. *Sharpey's* fibres are also found in bone in large numbers.

Going inward we find, bordering on the cement, the dentine (*d*) with its **Tomes' granular layer**, which we have already met (*tok*). *Tomes'* granules are nothing more than very minute, closely aggregated interglobular spaces. The dentine canaliculi of the root show somewhat different conditions than those of the crown. Their course is undulating and sometimes abruptly broken off. Dichotomous division is common. They also lie closer together and have more branches in this portion of the tooth. They end between the *Tomesian* granules either blindly or anastomosing with others. Not infrequently we can observe how such a tubule traverses the cement border, running into the process of a cement canaliculus.

Fig. 106.—Enamel Prisms from a Human Wisdom Tooth

300. Isolation specimen. Chromic-osmic-acetic acid.

Isolated enamel prisms may be obtained by decalcifying a tooth in the manner which will be discussed under the preparation of the next specimen. We may also simply detach a small particle of the enamel with a needle, distribute it in a drop of levulose and cover the specimen with a cover-glass.

Enamel Prisms.

Medium power will reveal numerous, fibrelike formations of different lengths and measuring about 5 μ in thickness, the **enamel prisms**. Of course, we see here only fractions of enamel prisms, which really wind their tortuous way through the entire thickness of the enamel. On their surface

¹ Sharpey, English anatomist, 1802-80.

we can recognize a transverse striation, probably due to the undulating surface of the prisms, which thus presents narrower and broader sections in alternation.

We may acquaint ourselves with their shape or transverse section by chopping a slightly larger particle of enamel with the razor; we will then find numerous cross-sections of prisms, irregular, pentagonal or hexagonal in form.

PLATE 44

**Fig. 107.—Longitudinal Section through a Human Wisdom
Tooth During its Eruption**

Fig. 108.—Sagittal Section through the Lower Lip of a Child

Fig. 107.—Longitudinal Section through a Human Wisdom Tooth During its Eruption

300. $\frac{3}{4}$. Chromic-osmic-acetic acid. Frozen section. *Biondi* solution.

The soft parts of the tooth can be studied to best advantage in the decalcified specimen, preferably one from a youthful individual. They must be recently extracted, and are split longitudinally by a pair of strong pincers. If this is done carefully the pulp will be intact, at least on many places. The two halves of the tooth are suspended on threads in a corked flask containing 50 cm³ of chromic-osmic-acetic acid (p. 30). The fluid is renewed on the second and fifth days, and the tooth will be decalcified sufficiently after six to seven days to allow of the making of a large number of very thin sections on the freezing microtome. The cuts should be made so that they include the pulp. Before cutting, the tooth must, of course, be washed in running water for twenty-four hours. The sections are stained in *Biondi* solution (p. 67).

The Pulp of the Tooth.

Our picture shows a part of such a section. On the left we recognize the **pulp tissue**, a finely fibrillated connective tissue, containing few branching cells. Numerous blood capillaries and nerves are found. In the interior of the pulp the medullated nerves form a plexus and leaving the trunk they lose their sheath and penetrate between the odontoblasts, where they end. More toward the right the pulp is bounded against the dentine by moderately long cells, which are arranged in several layers. The outermost cells are long and cylindrical. They are closely approximated, like epithelium. The deeper cells are irregular and merge into the connective tissue of the pulp without any defined boundary. The outermost cells, arranged like epithelium, are the **odontoblasts**, the cells from which the dentine is formed; they send numerous fine processes toward the pulp, which are lost in the connective tissue of the pulp; toward the dentine they send one or possibly two long processes, which enter the dentine tubules, where as **dentine fibres** or **Tomesian fibres** they are distributed throughout the dentine.

Between odontoblasts and dentine a cleft is seen, produced by shrinking of the pulp during fixation. In the dentine we have two sharply divided layers, which are separated by an undulating line. The bulk of the dentine has taken a violet color, only the innermost stratum, bordering on the odontoblasts, has stained purely red. The latter we call prædentine; it is that portion of dentine in the basic substance of which lime salts have not as yet been deposited.



Fig. 107.

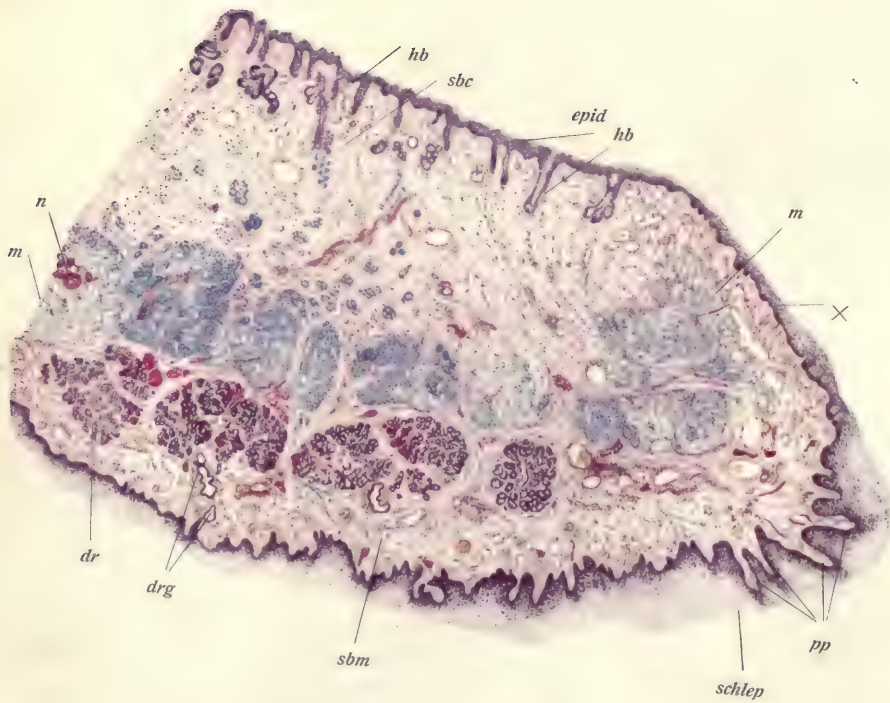


Fig. 108.

Fig. 108.—Sagittal Section through the Lower Lip of a Child

15. $\frac{3}{4}$. Formalin. Frozen section. Cresyl violet.

Using sharp scissors we cut, in sagittal direction, strips 5 mm in width from the lower lip of the fresh body of a child; they are fixed in formalin. Frozen sections are stained with cresyl violet (p. 62), which will give a good differential coloring of all the tissues in question.

In Fig. 108 the outer surface of the lip looks upward, the inner surface downward, and the free edge of the lip is at the right. All these surfaces are clothed in epithelium, so that we find **epidermis** (*epid*) above and **epithelium of the oral cavity** (*schlep*) below. Both are stratified epithelia, but present characteristic differences. The epidermis of the lip consists of relatively few layers of cells; the underlying connective tissue corium forms very slight papillæ. Externally to the violet epithelium we find a very thin pale layer, which represents the horny portion of the epidermis. A different picture presents itself in the epithelium of the mucous membrane (*schlep*). We first notice its great size. It consists of many more strata of cells than did the epidermis and, while there is some horny material in the most superficial layers, there are no non-nucleated horny scales, thus keratosis is incomplete. The connective tissue *propria*, following further upward, viz., away from the oral cavity, forms numerous **papillae**, sending secondary processes into the epithelium, the papillæ growing higher as we approach the free edge of the lips; in our specimen they must have been cut slightly obliquely, appearing a trifle larger than they really are. Tracing our way toward the right, around the edge of the lip, we come to a few papillæ in the now thinner epithelium, which have been cut off their bases and finally to a place marked X, where the mucous membrane merges into the epidermis.

Subcutaneous Tissue.

Embedded in the corium of the lip are many **hair follicles** (*hb*), from which the cut *hairs* project. **Sebaceous** and **sudoriferous** glands are likewise found. The subsequent subcutaneous tissue (*sbc*) of the lip contains besides a moderate amount of blood-vessels and red-stained nerves, small groups of transversely and longitudinally cut striated **muscle fibres**. The longitudinal muscular fibres have been collectively described as the compressor labii muscle.

Muscular Layer.

Between skin and mucous membrane of the lip we find a muscular stratum (*m*), consisting of numerous bundles of transversely cut, striated muscle fibres; they are the bundles of the **orbicularis oris**. The anterior extremity of the muscular band is bent at an angle, disappearing as a tapering strand at the region of the junction of mucous membrane and epidermis. The muscular bundles are often separated by the longitudinal fibres of the compressor labii, running from the skin to the mucous membrane. In the

anterior portion of the muscle we also find quite large, transversely cut blood-vessels, branches of the inferior labial artery and vein.

Submucosa.

Following this we have the submucosa of the mucous membrane of the lip, wherein we find, situated closely under the muscular layer, a thick glandular stratum (*dr*). The **labial glands** are distributed in large groups, separated by connective tissue. Their ducts (*drg*), mostly cut obliquely, are directed toward the epithelium, finally opening into the oral cavity. The nature of these glands may be recognized by the result of our stain. Within the reddish-violet glands we find here and there purely red portions. This is indicative of **mucus**. The glands of the lip have a mixed function, producing both mucus and albumin.

A glance over our specimen convinces us that submucosa and propria are by far richer in blood-supply than the subcutaneous tissue and the corium of the skin. We may see vessels and their yellow contents even in the papillæ of the propria.

PLATE 45

Fig. 109.—Dorsum of the Human Tongue

Fig. 110.—Base of the Human Tongue

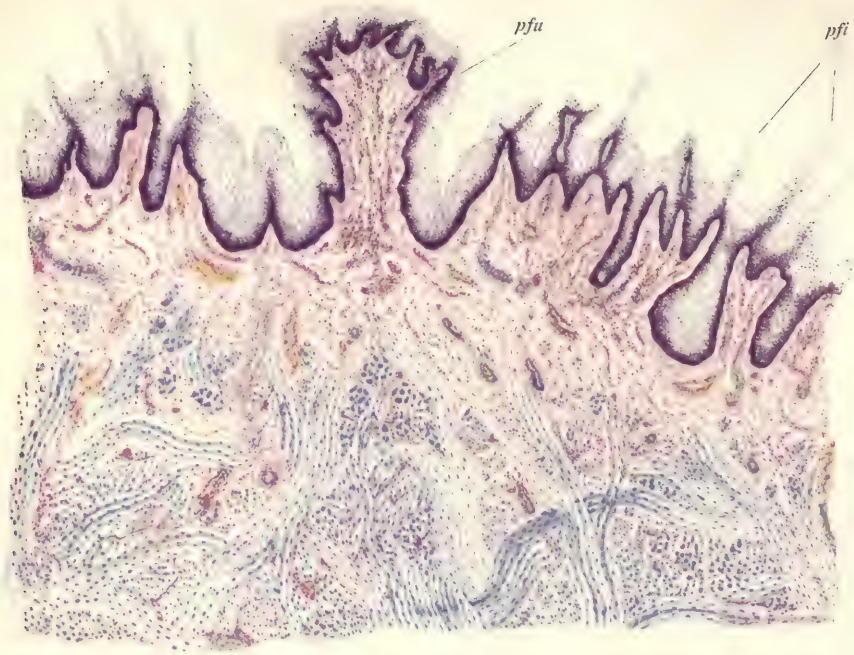


Fig. 109.

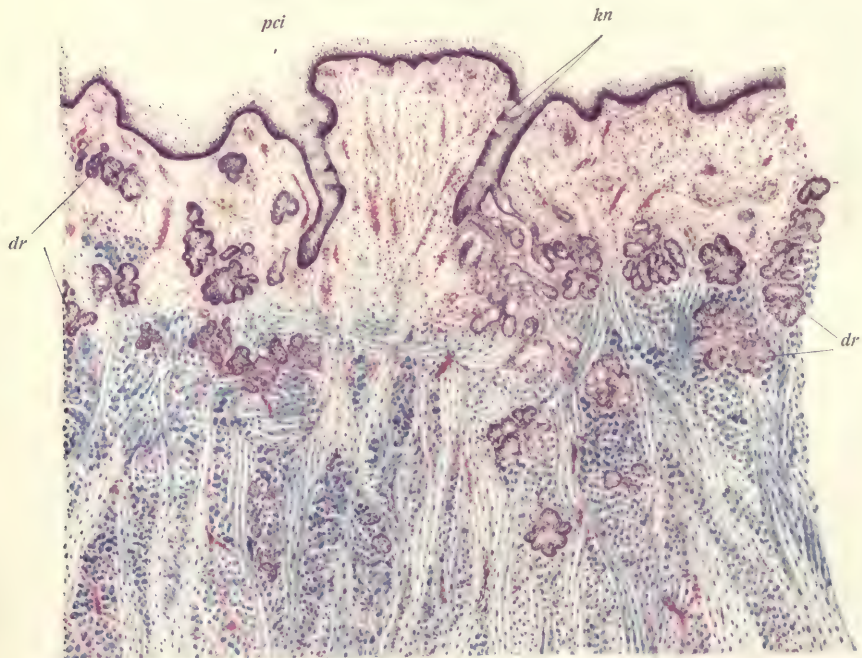


Fig. 110.

Fig. 109.—Dorsum of the Human Tongue

35. $\frac{3}{4}$. Formalin. Frozen section. Cresyl violet.

The tongue of an adult is fixed *in toto* in a large amount of 10% formalin, after having made numerous deep incisions at intervals of about 5 mm with the razor previously. The following day small pieces are excised from the various regions, transferred into 5% formalin, and afterward sectioned on the freezing microtome. Sections are stained in cresyl violet (p. 62).

We choose a section through the anterior portion of the dorsum of the tongue, where we find, with low power, the entire surface covered by elevations, the **papillae of the tongue**. In our section we are able to distinguish two varieties, the **filiform** and the **fungiform** papillæ, only one of the latter being present.

Filiform Papillæ.

Each filiform papilla (*pfi*) arises as a more or less broad body from the connective tissue **propria** of the mucous membrane of the tongue. The body either ends in a pointed extremity or, becoming broader, divides into a larger number of **secondary papillae**. Thus we may have as many as twenty secondary papillæ implanted on one papillary body. The body of the papilla consists of connective tissue, and contains numerous vessels and nerves. The surface of the papillæ is covered by the same stratified flat epithelium, as we have met in the mucous membrane of the lip. The epithelial stratum is frequently drawn out into fringed points and threads, which give a gray appearance to the superimposed mucous membrane. The cells, composing the threads, are keratosed.

Fungiform Papillæ.

In the centre of our specimen a fungiform papilla (*pfu*) is seen. It has a more slender body, which is also studded with numerous secondary papillæ, which are, in contradistinction to the filiform papillæ, covered by a smooth layer of epithelium. The outer cells do not show any keratosis, wherefore the surface of these papillæ do not appear gray, but red, the color of the translucent blood.

Muscles of the Tongue.

Following the **propria** we have a very thin connective tissue **submucosa**, which connects the mucous membrane with the **muscles of the tongue**. We find bundles of striated muscle fibres, which intersect, viz., antero-posterior bundles, cut transversely in our section, those running from above downward cut longitudinally in our specimen, and finally those going

from left to right also cut longitudinally. The bundles traversing the body of the tongue vertically are derived from the genioglossus, the hyoglossus and the verticalis linguæ, ending in the submucosa.

Fig. 110.—Base of the Human Tongue

35. $\frac{3}{4}$. Formalin. Frozen section. Cresyl violet.

For the study of the circumvallate papillæ we select a piece of the base of the tongue, where, as we know, these papillæ form a V-shaped design.

Circumvallate Papillæ.

They assimilate the fungiform papillæ in structure, but are considerably broader, also reaching deeper into the mucosa, which latter forms a **dam** around the papilla. The dam is separated from the latter by a deep trench. Into this ditch open numerous **glands** (*dr*), which are partly situated in the submucosa, partly deeply between the muscular bundles. As shown by their staining, they are serous glands. They are only found in the immediate vicinity of the circumvallate papillæ and the so-called papilla foliata, situated near by.

The epithelium of the circumvallate papillæ does not materially differ from that of the fungiform papillæ, but is characterized by a large number of **taste-bulbs** (*kn*). In the fungus-shaped papillæ they are present in very small amounts. In the circumvallate papillæ they are found in dense groups within the trench. Our specimen shows them as oval formations, situated within the epithelium, their long axis forming a right angle with the surface. Their structure will be discussed in detail under the organs of special sense (p. II, 375).

The muscles are arranged in the same manner as in the preceding specimen.

PLATE 46

Fig. 111.—From a Section of the Human Faucial Tonsil

Fig. 112.—Sublingual Gland of Man





Fig. 111.

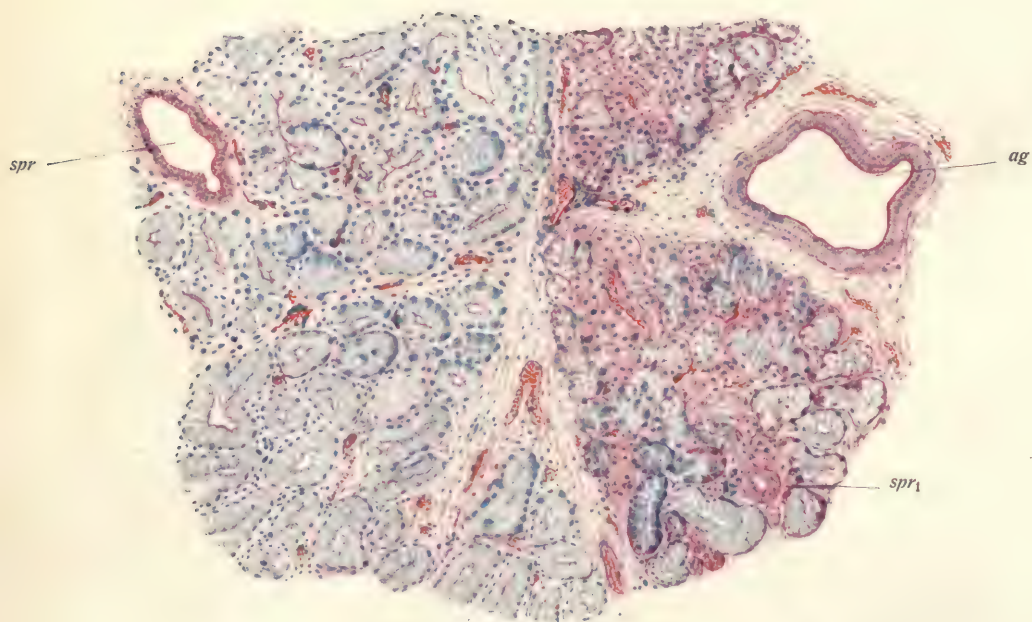


Fig. 112.

Fig. 111.—From a Section of the Human Faucial Tonsil

24. $\frac{3}{4}$. Formalin. Frozen section. *Biondi* solution.

The excised organ is divided longitudinally into two halves, which are fixed in 10% formalin. Frozen sections are made and stained in *Biondi* solution (p. 67).

The Tonsil.

Our picture shows the initial portion of the specimen. At the left we see the mucous membrane of the mouth and its minute mucous glands (*schldr*), below that the bisected muscles of the faucial fold. As the mucous membrane approaches the tonsil, the number of **lymphocytes** in the propria increases, gradually forming small **follicles** (*fo*). The surface of the organ itself shows a deep sinus, caused by the *tonsillar fossulae*, one of which is represented here (*ft*). The epithelium of the oral cavity (*ep*) continues into these sinuses, subject to certain changes (*ep₁*). While in the oral cavity it is sharply defined from the propria, the border-line here is not so distinct, due to the presence of lymphocytes in both propria and epithelium. The entire wall of the sinus consists of adenoid tissue, such as we have met when discussing the lymph-gland (p. II, 149). Smaller and larger follicles (*fo₁*) are found within it. The lymphocytes furnished by them migrate constantly through the epithelium of the sinuses into the oral cavity, thereby altering the appearance of the former.

Fig. 112.—Sublingual Gland of Man

60. Sublimate. Frozen section. *Biondi* solution.

For the study of the large salivary glands small pieces of the gland of an adult are excised shortly after death and transferred for five to six hours into a 3% sublimate solution. They are washed in running water overnight and thereafter placed in 5% formalin. Very thin frozen sections are stained in *Biondi* solution (p. 67).

Under low power we find the gland arranged in **lobules**, i.e., smaller or larger parts of glandular parenchyma are separated by connective tissue. Within the interlobular connective tissue we see, aside from the large vessels, the glandular **ducts**. They are of varying calibre and show different structures. The large ducts (*ag*), which either join to form the ductus sublingualis major or leave the gland as ductus sublinguales minores, are lined by a double layer of cylindrical epithelium. Further inward we have a layer of moderately high cylindric cells, covered by a bright red cuticular

band, underneath which we see a single layer of cuboid cells. Externally a structureless basal membrane follows and, according to the size of the duct, a greater or lesser amount of connective tissue, with a few smooth longitudinal muscle fibres intermingled.

These large ducts are formed by the union of smaller ducts, which are likewise interlobular; one of these is depicted at the left side of our specimen (*spr*) in transverse section. These **salivary tubules**, as we will call them, differ from the ducts primarily by their epithelium. Instead of two layers we have one single layer of cylindric epithelium. Furthermore, the connective tissue adventitia of the duct has been considerably reduced in size.

Intralobular Salivary Tracts.

The branches of the salivary tubules now enter the lobules themselves, forming the **intralobular tracts**, which differ in structure from the former inasmuch as the cells become flatter. One such tract is seen at *spr*₁, and here we can observe its further destination. It becomes abruptly thinner, merging into the short **inserted piece**, which is lined by low cuboid cells and in turn breaks up into several cylindrical or more rounded spherical **end-pieces**. The latter are lined by the actual **secretory cells**.

The Secretory Epithelium.

As regards the secretory cells, the different lobules show variations. One lobule will be found to be composed exclusively of blue **mucous cells**. Here the cells are fairly large, the end-pieces are cylindrical, having wide lumina and showing numerous lateral bights. Close by we may encounter a lobule which, aside from the blue mucous cells, contains numerous red cells, which either lie between the mucous cells or form the lining of the end-pieces exclusively. High power will demonstrate to us that in many mucous cells the central portion alone contains mucus, while the base of the cell, where the nucleus is situated, holds red-stained protoplasts. In these lobules, which are evidently in the state of secretion, we may study all the stages of the expulsion of mucus. The cell, when filled with mucus, appears large, staining light blue in our specimen. Its nucleus lies at the base, being compressed and flattened by the pressure of the contents. As soon as the expulsion of mucus sets in, the nucleus will resume its round shape, the red protoplasm around it reappearing and advancing toward the lumen until all the mucus has been expelled. As the mucus is thrown off the cell decreases in size considerably.

The sublingual gland of man thus is to the greater part a mucous gland, although we find aside from those end-pieces, which are lined by mucous cells, also such which contain **albumin cells**.

PLATE 47

Fig. 113.—The Human Parotid Gland

Fig. 114.—The Human Submaxillary Gland

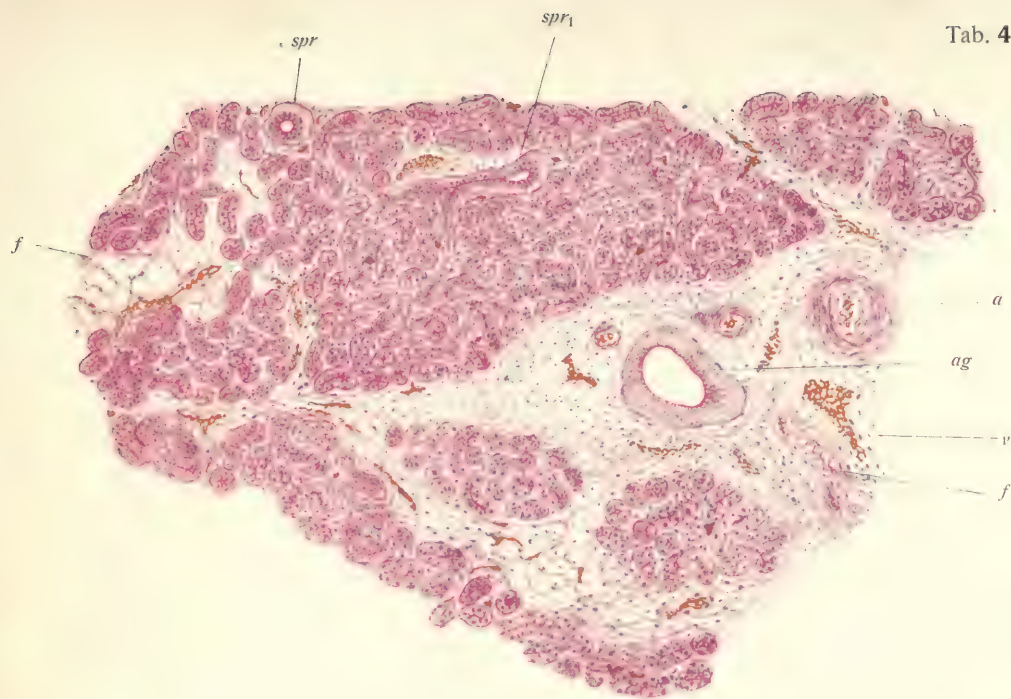


Fig. 113.



Fig. 114.

Fig. 113.—The Human Parotid Gland

60. Sublimate. Frozen section. *Biondi* solution.

The parotid gland, though similar in many respects to the sublingual, contains no mucous glands. It is likewise a lobulated gland; the lobules are separated by still stronger connective tissue bands, containing fat (*f*). The ducts do not differ materially from those of the sublingual gland; *ag* shows such a duct, lined by stratified cylindrical epithelium and surrounded by arteries (*a*) and veins (*v*). A different picture is met in the salivary tubules. *Spr* shows the transverse section of such a tubule. The cells are relatively high, presenting a dark inner and light outer zone, the latter having a fine radiating striatum. We have here **Heidenhain's rod-cells**, the details of which we will discuss later. The transition of these salivary tubules into the secretory end-pieces takes place through long, inserted pieces, see at *spr*₁, to which the small rounded or elongated end-pieces are attached.

Secretory Epithelium.

The epithelium lining the latter is composed of small cuboid or conical cells, the finer types of which we have already met with at the beginning of our course (p. II, 16). These granules, which we observed at that time in the meshes of the cellular protoplasm, represent the pre-stage of the parotid secretion, an albuminous, clear, freely flowing liquid, while the secretion of the sublingual gland is viscid, due to its mucous contents. Thus we can say that the human parotid is a **purely serous gland**.

Fig. 114.—The Human Submaxillary Gland

60. Sublimate. Frozen section. *Biondi* solution.

The submaxillary gland of man shows striking structural similarities with the parotid, differing from it by the fact that, besides the globular serous end-pieces, we also find elongated mucous end-pieces. The former are much more numerous in man than the latter. The interlobular connective tissue is never so strongly developed as in the parotid. Our specimen shows in this interlobular connective tissue besides arteries (*a*) and veins (*v*), also a small **duct** (*ag*) which divides above to form **salivary tubules**, the lumen of the duct becoming wider thereby. The left branch (*spr*₂) merges quickly into a short **intermediate piece**, to which is attached a short end-piece lined by albumin cells. Similar conditions prevail in the salivary tubules *spr*₁ and *spr*₃, with the difference that here the intermediate pieces are longer

and studded with several end-pieces. A cross-section of a salivary tubule is seen at the place marked *spr*, showing the wealth of this gland in tracts, lined by rod epithelium, as compared with the parotid.

*Mucous Tubules and
Gianuzzian Crescents.*

Sharply defined from the red-stained serous end-pieces are the blue mucous tubules, which are connected with the salivary tubules by intermediate pieces in the same manner as the serous end-pieces. The cells are perhaps a trifle smaller than those of the sublingual gland. On the free end of the mucous tubule we always find a group of albuminous cells, arranged like a hood, the so-called **Gianuzzian crescent**.

PLATE 48

Fig. 115.—Submaxillary Gland of Man

**Fig. 116.—Injected System of Ducts in the Submaxillary Gland
of the Cat**

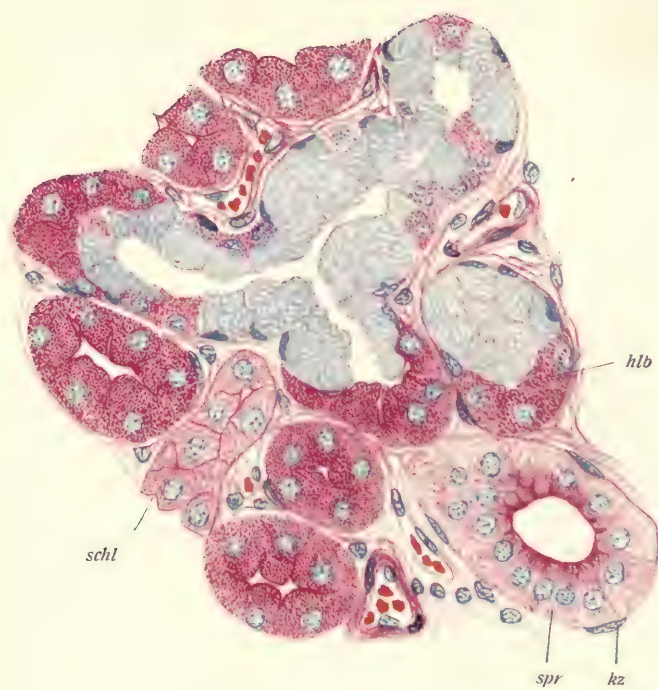


Fig. 115.

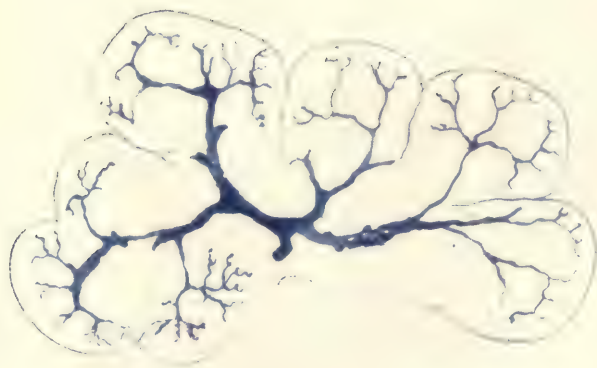


Fig. 116.

Fig. 115.—Submaxillary Gland of Man

400. Sublimate. Frozen section. *Biondi* solution.

Finer Structure of the Submaxillary Gland.

We will now take one of the sections, select a proper field and examine it with high power. First we notice a *salivary tubule*, which has been cut obliquely. Externally it is surrounded by a red, structureless **membrana propria**, on the inner side of which we observe nuclei (*kz*). They belong to the **basket cells**, branching cells, the processes of which anastomose; they are deposited between the membrana propria and the epithelial cells. They are found on the secreting end-pieces as well as on the intermediate pieces, while on the salivary tubules they gradually disappear. The epithelium of the salivary tubules consists of cylindric cells, the outline of which can barely be discerned. The cement plugs, interposed between the free ends of the cells near the lumen, render the outline of the individual cell visible. The nucleus lies in the cell centre or nearer to the lumen of the tubule. The basal end of the cell shows the rod design, as mentioned before. The microsomes of the protoplasm are arranged successively in rows, giving the appearance of rods in thicker sections.

An **inserted** or **intermediate piece** (*schl*) is also seen in our section; however, it has been cut obliquely, so that the lumen with its lining of light, low, cylindrical cells can only be seen at one place.

Several **serous end-pieces** are seen, partly in oblique, partly in transverse section. Their lumen is narrow; the conical cells are filled with bright red secretory granules. The nucleus generally lies near the base of the cell, but may advance as far as the centre. The lumen sends out minute processes between the cells, the **secretory capillaries**.

Finally we will examine the **mucous end-pieces**, of which we find two sections in our specimen. Their widely gaping lumen is lined with mucous cells of different sizes, the average being larger than the serous cells. They are filled with mucous granules; the nucleus is compressed, lies at the cell-base, and frequently is jagged, presenting pointed processes. Between the cells, filled with mucus, we also find such as have partly or entirely given up their contents.

Three **creasents** or **halfmoons** (*hlb*) are shown in our specimen. The one in the centre is most favorably cut. We will notice how the crescent cells are the direct continuation of the mucous cells; they also spread sideways between the mucous cells and the membrana propria, pushing the former away from the latter. From the lumen of the end-piece, near the crescent, several minute secretory capillaries arise, which advance between the crescent cells and along the border-line between mucous and crescent cells. The cres-

cent cells themselves show the same structure as the cells of the serous end-pieces, being, without doubt, identical with them.

Fig. 116.—Injected System of Ducts in the Submaxillary Gland of the Cat

300. Vital injection of methylene blue. Frozen section.

To gain a general aspect of the system of ducts draining this labyrinth of glandular tubules and aceni, we may use the injection method, introducing Berlin blue through the duct. Such an injection into a blind system of ducts is very difficult, and naturally is very apt to result in extravasation, hence the natural injection is much to be preferred. A 1-2% solution of methylene blue should be used in the manner described on p. 59. The experiment can be conveniently made on the cat. A quarter of an hour after the completion of the injection the gland is excised, divided into small pieces, and the latter are fixed and washed in the manner described on p. 62. Thick sections are made on the freezing microtome, quickly dehydrated in alcohol and mounted in balsam.

The activity of the gland has lasted through the injection; the dye having been excreted by the cells, now occupies the lumen of the ducts, which appear filled with the dyestuff, while the cells themselves remain absolutely unstained. In other places the reverse condition prevails, the ducts being empty and the cells, which evidently lost their vitality early, being stained blue. The nuclei can, of course, be stained separately in such a section with any desired dye, e.g., carmalum, but in a specimen of this character such a procedure is hardly advisable.

The submaxillary glands of the cat and the dog are mixed glands, but, in contradistinction to the human submaxillary, the mucous element predominates by far, the serous portion being only represented by the crescents. Our specimen shows a mucous end-piece, dividing into seven branches. A crescent is mounted upon each end-piece; between the cells of the former we can notice the terminal branches of the duct system ending in secretory capillaries.

PLATE 49

**Fig. 117.—Longitudinal Section through the First Portion of the
Infantile Oesophagus**

**Fig. 118.—Longitudinal Section through the Cardiac Orifice of
the Stomach of the Monkey**

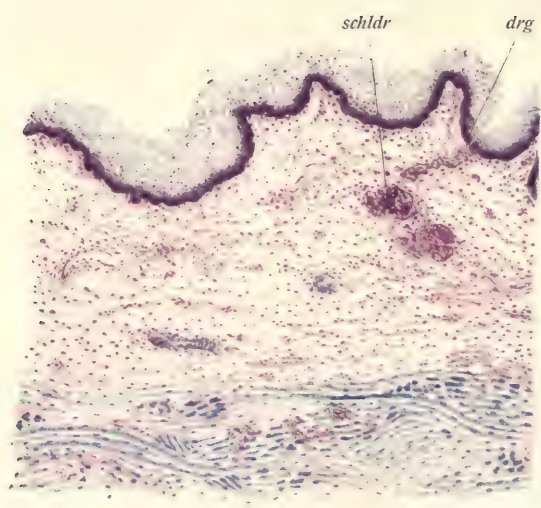


Fig. 117.

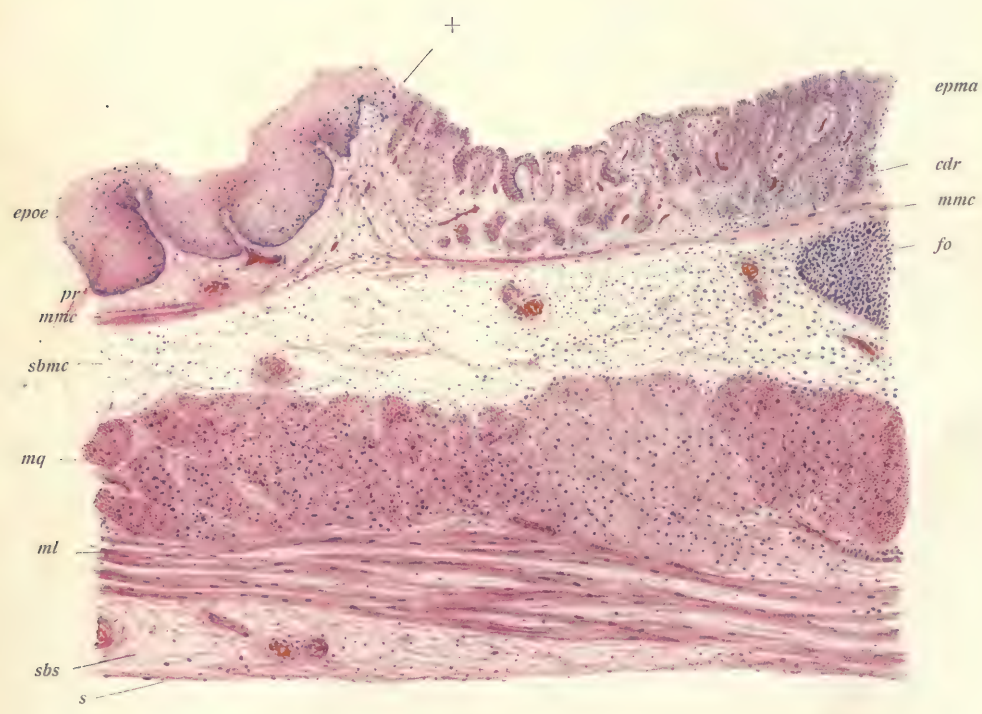


Fig. 118.

Fig. 117.—Longitudinal Section through the First Portion of the Infantile Oesophagus

35. $\frac{3}{4}$. Formalin. Frozen section. Cresyl violet.

Little is to be said about the technical manipulation of the specimen. The oesophagus is cut open longitudinally, mounted on a wax plate and fixed in 10% formalin. The following day small strips are cut with the razor at different heights, transferred to 5% formalin and sectioned the next day on the freezing microtome. The sections are stained in cresyl violet (p. 62).

Oesophagus.

Our picture represents a section from the beginning of the oesophagus, cut longitudinally. The **epithelium** is the stratified epithelium of the oral cavity. It is mounted on a **propria**, which, like the one of the mouth, forms papillæ. The **submucosa** here is still intimately connected with the propria, becoming separated from it lower down. In the submucosa we find a few **mucous glands** (*schldr*), their duct being cut longitudinally (*drg*) for a short distance. Furthermore we find, in other sections, a different sort of glands, situated more superficially, the so-called **cardiac oesophageal glands**, which we will meet again when discussing the cardiac orifice of the stomach.

The muscular layer of the oesophagus consists in the upper third almost exclusively of striated fibres, which form bundles, running longitudinally and in spiral fashion; they appear therefore in longitudinal and oblique section. In the middle portion of the oesophagus the striated fibres are more and more replaced by smooth muscle, which exclusively forms the muscularis of the lower third.

Fig. 118.—Longitudinal Section through the Cardiac Orifice of the Stomach of the Monkey

35. $\frac{3}{4}$. Sublimate-acetic acid. Frozen section. *Biondi* solution.

Since it is very difficult to obtain absolutely normal human gastric mucous membrane, we will confine ourselves to animal material. Monkey, cat and dog furnish excellent specimens, which essentially present the same conditions as found in man. The animal should be starved for one to two days and killed with chloroform. The stomach, including a part of both oesophagus and bowel, is excised immediately after death, spread on a large wax plate and fixed in sublimate-acetic acid (p. 31) for five to six hours. After washing, we excise narrow pieces from the different parts, cardia, fundus and pylorus, which are transferred to 5% formalin and cut the following day on the freezing microtome. Cresyl-violet (p. 62) proves the most valuable

preparation for staining. In the most simple manner possible it brings about an excellent differentiation of all the important elements. *Biondi* solution (p. 67) is also to be recommended.

*Transition of the
Œsophagus into the Stomach.*

We will first consider a longitudinal section through the cardia of the monkey, which will demonstrate to us the transition (X) of the œsophagus into the stomach. On the left we have the extensively stratified flat epithelium of the œsophagus, which becomes thinner toward the right and ceases abruptly. Its place is taken by the much thinner simple cylindrical epithelium of the stomach.

The **propria** of the œsophagus (*mc*), forming high papillæ, is separated from the underlying **submucosa** by a thin layer of muscle, the **muscularis mucosae** (*mmc*). Although we meet it here for the first time, it is found in the higher sections of the œsophagus also. Where the mucous membrane of the œsophagus merges into that of the stomach, the propria forms a swelling, into which the fibres of the muscularis mucosæ radiate from both sides. This place may be recognized macroscopically as a ring-shaped fold projecting into the interior of the stomach. The epithelium of the stomach dips into the propria on various places, forming numerous glands, which we call **cardiac glands**. They are, as we have seen, present in the œsophagus. In the stomach they are found only in the region of the cardia, soon giving way to the fundus glands. They are in all essentials similar to the pyloric glands, which we will soon discuss, having, like the latter, a lining of light cylindrical cells.

In the connective tissue submucosa (*sbmc*) we notice at one place a collection of lymphocytes, a small follicle (*fo*). Such follicles are not rare in the submucosa and mucosa of the human stomach; whether they should be considered normal or pathological is a question.

The **Smooth Muscle Layer** of the œsophagus is continuous with that of the stomach. We find an outer, thin longitudinal layer, followed internally by a thicker circular stratum. In other parts of the stomach we have, in addition to these, some oblique bundles.

Externally to the muscularis is the strongly developed, vascular **subserosa** (*sbs*), a loose connective tissue layer, containing a network of elastic fibres. Following this layer we have the **serosa** (*s*), a simple flat epithelial stratum, such as we have met before. It is separated from the subserosa by an elastic membrane.

PLATE 50

Fig. 119.—Mucous Membrane of Fundus of Monkey's Stomach

Fig. 120.—Fundus Gland of the Monkey

Fig. 121.—Pyloric Mucous Membrane of the Monkey

Fig. 119.—Mucous Membrane of Fundus of Monkey's Stomach

200. $\frac{3}{4}$. Sublimate-acetic acid. Frozen section. Cresyl violet.

Looking at a section of the fundus of the stomach with low power, we recognize narrow depressions in the mucous membrane, which can be distinguished macroscopically, being known as **gastric dimples** (*magr*).

Surface Epithelium of the Stomach.

These depressions are lined with the surface epithelium of the stomach. The latter consists of a simple layer of long cylindrical cells. The nucleus is most often found near the base within deep-staining granular protoplasm. The rest of the cell stains very weakly. These cells are credited with the secretion of the gastric mucus. Treated with reagents as well as with dye-stuffs, they differ, however, distinctly from other mucous cells, e.g., the goblet cells or the mucous cells of the salivary glands. Our specimen shows this plainly. While the ordinary mucous cell takes a metachromatic red stain with cresyl violet, the gastric epithelial cell, like other protoplasm, stains blue. Perhaps this is due to a greater percentage of albumin in these cells.

Fundus Glands.

The **gastric glands** open in the depth of the gastric dimples. In the fundus they are long, simple or compound tubular glands, generally very closely aggregated. They are surrounded on all sides by the propria of the stomach, the latter rising between them to form the foundation for the walls of the dimples. The propria always contains mast-cells (*mz*) and smooth muscle fibres (*m*) arising from the muscularis mucosæ, which is not included in our specimen. Furthermore, it is supplied with a dense network of blood capillaries, which encloses each gland and reaches up to the surface epithelium.

Each gland presents a **neck** (*dth*), which opens into a gastric dimple, an elongated **body** (*drk*) and a blind **fundus** (*drgr*), the latter often somewhat bent. The lumen is fairly narrow and lined by two kinds of cells. Some are smaller and stain reddish violet, others are larger and stain blue. The former are the **chief cells**, the latter the **parietal cells**.

Distribution of Chief and Parietal Cells.

These two kinds of cells are not evenly distributed over the glandular tube. In the neck the parietal cells predominate, becoming scarcer toward

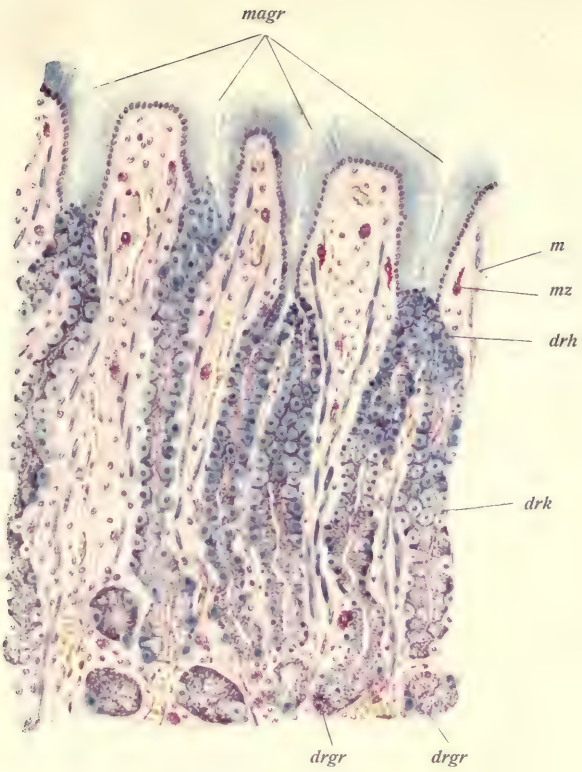


Fig. 119.

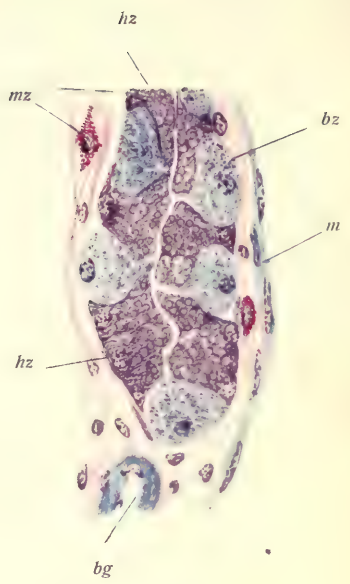


Fig. 120.



Fig. 121.

the fundus. While in the neck they lie between the chief cells; they deserve their name better in the glandular body, where they form the parietes of the tube.

Fig. 120.—Fundus Gland of the Monkey

600. $\frac{3}{4}$. Sublimate-acetic acid. Frozen section. Cresyl violet.

Examining the base of such a gland under high power, we find characteristic differences between the two varieties of cells. The glandular tube is surrounded by a structureless *membrana propria*, studded externally by nuclei, which belong to cells of a shape similar to that of the basket cells of the salivary glands (p. II, 193).

Chief Cells.

Here the narrow lumen of the gland is lined exclusively with chief cells, high cuboid or low cylindric cells, the bodies of which are entirely filled with coarse granules. It is often difficult, at times impossible, to define the borders of the different cells. The granules stain in the same reddish violet hue as the nucleus, which lies at the base of the cell and is easily overlooked.

Parietal Cells.

These are essentially larger than the chief cells; they are more or less remote from the lumen of the gland, being connected with it by shorter or longer ducts, leading between the chief cells to the lumen. The cell-body is light blue and filled with numerous fine granules, which are stained dark blue. The round or oval nucleus lies either in the centre of the cell or may be situated more toward the base. Not infrequently we find, especially distinctly in the uppermost parietal cell, that the cell-body is crossed by a network of light lines. They are the so-called *basket capillaries*, a meshwork of tubules within the cells, through which the secretion leaves the cell. The basket capillaries are only found in the parietal cells, not in the chief cells.

Fig. 121.—Pyloric Mucous Membrane of the Monkey

200. $\frac{3}{4}$. Sublimate-acetic acid. Frozen section. Cresyl violet.

A picture differing entirely from the mucous membrane of the stomach is presented by the mucous membrane of the pylorus. Our specimen shows very roomy and deep gastric dimples (*magr*). In man they are as deep, but not quite so broad. They are lined with the same cylindrical epithelium as those of the fundus.

Pyloric Glands.

The gastric glands here are quite different from those seen before. They are not so closely approximated, being separated by greater interspaces, oc-

cupied by the propria. Again they are long simple or compound tubules like those of the fundus, but they are not placed longitudinally, but have a tortuous course, so that we do not find longitudinal, but transverse and oblique sections of the tubule.

The glands are lined with cuboid or cylindrical cells, which completely assimilate the chief cells of the fundus gland, containing likewise varying amounts of coarse secretory granules.

The chief cells thus are a common element of the glands of all parts of the stomach; they have, in all probability, the function of secreting the ferment of the gastric juice, the **pepsin**. The granules contained within them may be regarded as a pre-stage of it. Our specimen shows granular masses in the dimples of the pylorus, after having been expelled from the cells. The parietal cells, on the other hand, are only found in company with the chief cells in the glands of the fundus. They probably are the manufacturers of the *hydrochloric acid* of the gastric juice.

PLATE 51

Fig. 122.—Junction of Stomach and Duodenum of the Monkey

Fig. 123.—Jejunum of Child

Fig. 122.—Junction of Stomach and Duodenum of the Monkey

Longitudinal section. 15. $\frac{3}{4}$. Sublimate-acetic acid. Frozen section.
Biondi solution.

Transition of Stomach into Duodenum.

The junction of stomach (*ma*) and intestine (*dd*), shown in our specimen, is marked by a fold of mucous membrane, which projects into the duodenum. Beneath it the powerful muscular layer forms a ring-shaped swelling, tapering toward the lumen, the **sphincter pylori** (*sphp*). It consists mainly of circular fibres, into which the longitudinal muscular fibres pierce from the exterior in a radiating arrangement.

At the left we see the pyloric mucous membrane with transverse and oblique sections of glands; it extends over the height of the fold and only at the distal declining surface of the fold merges into the intestinal mucous membrane. Just in front of the junction we see a small **lymph-follicle** (*fo*).

The intestinal mucous membrane (*mc*), even under low power, differs in many respects from the gastric mucous membrane. One differentiating factor is found in the presence of shorter or longer, fingerlike processes, projecting into the lumen, the **villi** (*z*), between which we notice depressions reaching down to the muscularis mucosæ (*mmc*), tubular in shape, the **glands** of **Lieberkuehn** (crypts of L.). Besides these we find a large deposit of glands in the submucosa, **Brunner's glands** (*brdr*). Contrary to the findings in the stomach, we thus have here glands which are situated in the submucosa. The surface epithelium likewise presents characteristic differences. While in the stomach, the cells stained in an even pale red, we find among the intestinal epithelium numerous elements staining intensively blue with the basic dye; they are **goblet cells**.

Fig. 123.—Jejunum of Child

Longitudinal section. 25. $\frac{3}{4}$. Formalin. Frozen section. *Biondi* solution.

For the study of other portions of the intestinal tract under low power we will select first the jejunum and later the transverse colon. Little need be said regarding the technique. The bowel should be excised shortly after death, split open longitudinally, immediately spread on wax plates and fixed in 10% formalin. Frozen sections are stained in *Biondi* solution (p. 67).

Structure of the Jejunum-Ilium.

Considering first a longitudinal section through the jejunum of the child, we find the intestinal mucous membrane in short folds, one of which presents

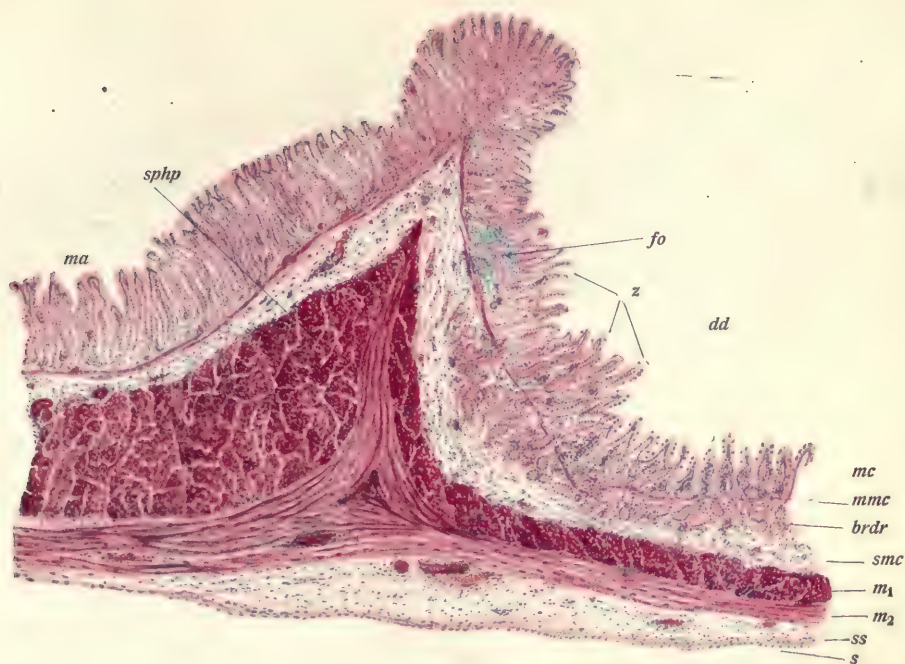


Fig. 122.

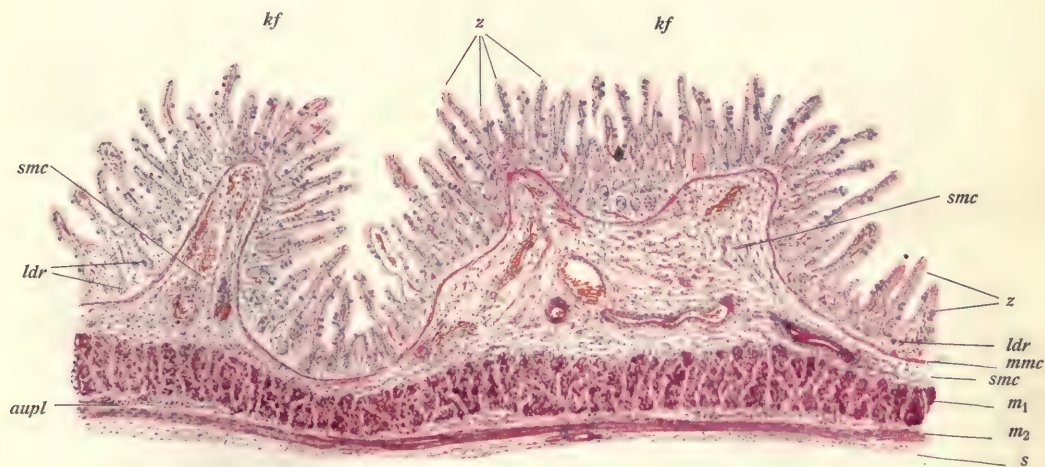


Fig. 123.

small secondary folds. They are **Kerkring's folds** (*kf*). They are covered by mucous membrane, which here has numerous long, fingerlike **villi** (*z*). Between the villi we again find the short **crypts** of **Lieberkuehn** (*ldr*), extending deeply into the muscularis mucosæ (*mmc*), which divides the mucous membrane from the submucosa. The **submucosa** (*smc*), well developed in the folds, is richly supplied with blood-vessels, but does not contain glands like the duodenum. The muscular coat again consists of an **inner circular** (m_1) and an **outer longitudinal** (m_2) layer. The main differentiating factors between jejunum and upper duodenum are therefore the presence of **Kerkring's** folds, the plicæ circulares and the want of **Brunner's** glands in the former. Between circular and longitudinal muscular layers we find the collection of nerves known as **Auerbach's plexus** (*aupl*). Externally the longitudinal muscular layer is covered by the **serous coat** (*s*).

PLATE 52

Fig. 124.—Transverse Colon of a Child

Fig. 125.—Villus from the Small Intestine of the Monkey

Fig. 124.—Transverse Colon of a Child

Transverse section. 35. Formalin. Frozen section. *Biondi* solution.

In the large intestine conditions vary essentially. First of all the circular *Kerkring's* folds, which had become rare in the lower part of the small intestine, disappear altogether. Their place is later on taken by the *plicae semilunares*, which, however, are not solely folds of the submucosa, but also contain muscularis.

The Muscular Coat of the Large Intestine.

The muscles of the intestine are also altered by the appearance of the *taeniae* (*t*). As shown in our specimen, they are but moderately developed in the bowel of the child, being represented by a mere thickening of the outer longitudinal muscular stratum. Between longitudinal (m_1) and circular (m_2) muscular layers we notice an interposed stratum in our specimen, the *nerve-plexus* of *Auerbach* (*aupl*). Viewed under high power (see Plate 23, Fig. 60), it appears composed of multipolar nerve-cells and non-medullated nerve-fibres, which are arranged in net form between the two muscular strata.

The submucosa presents no features of interest.

Mucosa of Large Intestine.

The mucosa again contains the *glands* of *Lieberkuehn* (*ldr*), the epithelium of which is extraordinarily rich in goblet cells. Our picture shows a *lymph-follicle* (*fo*) in the mucosa. The latter is for a distance transformed into lymphadenoid tissue, thus forming the follicle, into which the free surface of the bowel dips. This is characteristic of the follicles of the large intestine, but is never found neither in the simple nor the compound follicles of the small intestine.

The most important peculiarity of the mucosa of the large intestine lies in the fact that it does not form any villi. The epithelium shows in all essentials the same conditions as we will find in the small intestine. In the large intestine, too, mucosa and submucosa are separated by a muscularis mucosa (*mmc*), which, however, especially in the upper and middle portion of the large bowel, is less well developed than in the small intestine.

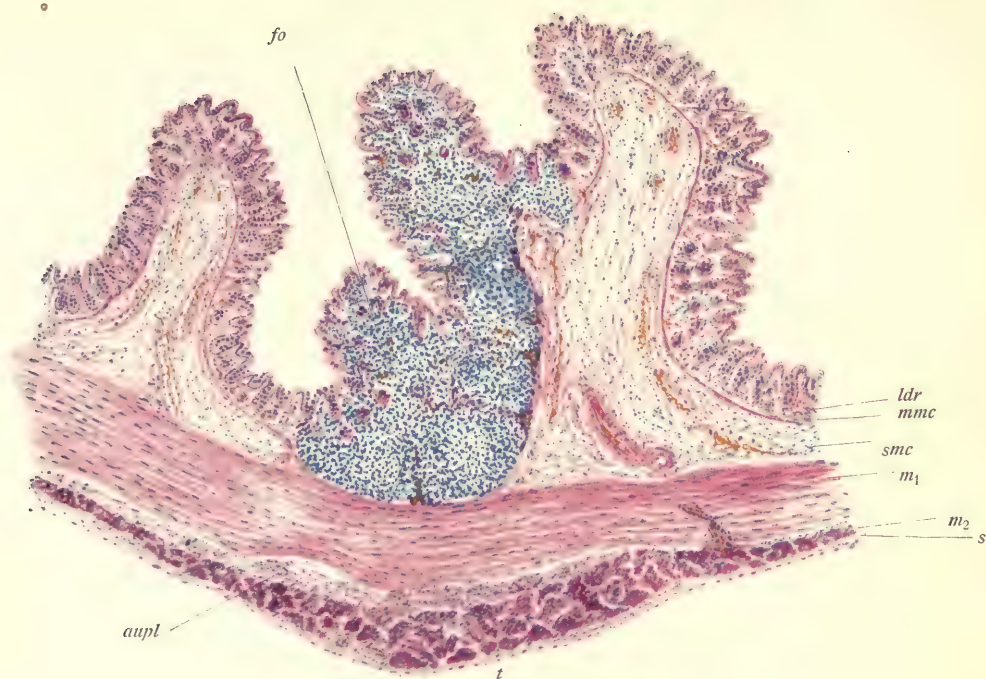


Fig. 124.

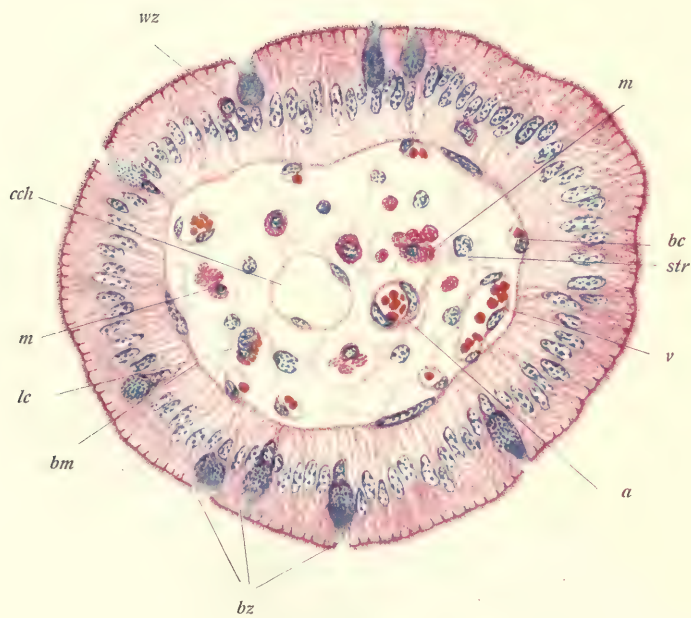


Fig. 125.

Fig. 125.—Villus from the Small Intestine of the Monkey

Transverse section. 400. $\frac{3}{4}$. Sublimate-chromic-osmic-acetic acid. Paraffin embedding. *Biondi* solution.

We will endeavor to make a detailed study of the structure of the intestinal villi. It proves quite difficult to obtain really good specimens of the same, since the action of many fixing agents will cause the body of the villus to become detached from its epithelial covering. This is especially true of our simple formalin fixation. The best results are obtained from osmic acid combined with sublimate. Of the various combinations we will select the sublimate-chromic-osmic-acetic acid (p. 31). The specimen must be fixed immediately after death; therefore we should use the intestine of the cat, dog or monkey. A piece of the jejunum is mounted on a wax plate in the aforementioned manner and placed in the above-named fluid for six to eight hours; thereafter it is washed in running water overnight and dehydrated in the usual manner. While in the 95% alcohol small square pieces are cut out with the razor, transferred to absolute alcohol, and after the use of chloroform are embedded in paraffin. Transverse sections through the villus are most demonstrative, but may be supplemented by longitudinal cuts. The sections are stained in *Biondi* solution (p. 67).

Structure of the Intestinal Villi.

Fig. 125 shows a section approximately through the centre of the villus. Externally the villus is invested with high cylindrical **epithelium**. Between the cylindrical cells numerous **goblet cells** (*bz*) are found. The structure of the epithelium has been exhaustively discussed under the topic of cylindrical cells (p. II, 35); we therefore refer the reader to that section. We again see **migrating cells** (*wz*) piercing the epithelium on two places.

The epithelium is mounted on the **body** of the **villus**, which latter is nothing more than an elevation of the intestinal mucosa. Externally it is covered by a thin **basal membrane** (*bm*). It consists of flat, branching cells, which are mounted upon a fine, homogeneous membrane, and send their processes into the **stroma** of the **villus**. The latter likewise consists of branching anastomosing cells, viz., reticular tissue (*str*). Numerous lymphocytes and leucocytes (*lc*) are found within the meshes. In our specimen they are surprisingly scarce.

The epithelium of the villus also encloses **blood-vessels**, arteries (*a*), veins (*v*) and capillaries (*bc*). The latter are generally superficial, being in close approximation with the basal membrane, while the artery, or rather arteries, at times two or three being present, are situated more deeply. The centre of the villus is claimed by a wide lumen, very favorably represented in our specimen; this is the **central chyle-vessel** (*lactile*). It mounts in the axis of the villus, being continuous in the base of the villus with a network of lymph-vessels, which is distributed over the intestinal mucosa and surrounds

the glands of *Lieberkuehn*. The central chyle-vessels of the villi thus represent the roots of a system of chyle-vessels.

The last component of the body of the villus is the **smooth muscle** (*m*). These fibres are found throughout the stroma of the villus as fine, bright-red cross-sections, which always distinctly show their composition of bisected fibrils. Now and then we obtain a glance at the quite narrow nucleus. Sometimes isolated cells are seen, but most always several unite to make a small bundle. These smooth muscle bundles are derived from the muscularis mucosæ. They cross the body of the villus in its long axis and become attached to the basal membrane. Their contraction will render the villus shorter, causing it to wrinkle in transverse folds. Such contracted villi are very frequently encountered in the transverse sections of the intestine of the dog and the cat.

PLATE 53

Fig. 126.—Glands of Lieberkuehn from the Intestine of the Child

**Fig. 127.—Small Intestine of Dog, Showing Injected
Blood-Vessels**

Fig. 128.—Human Liver

Fig. 126.—Glands of Lieberkuehn from the Intestine of the Child

400. $\frac{3}{4}$. Formalin. Frozen section. *Biondi* solution.

The study of the glands of *Lieberkuehn* is a good deal simpler than that of the villi. The epithelium is easily fixed with formalin, this method being especially favorable for the preservation of certain granular elements of the cells of the glands. Frozen sections, stained in *Biondi* solution (p. 67), will give excellent demonstrations.

Structure of the Glands of Lieberkuehn.

The **glands** or **crypts** of *Lieberkuehn* are either simple or, as our specimen shows, bifurcating crypts or depressions in the intestinal mucous membrane, varying in length in different portions of the gut. They are surrounded by a **membrana propria** (*mpr*), which is similar in structure to the basal membrane of the villi, being the direct continuation of the latter. The lining epithelium is very similar to the epithelium of the villi, but slightly lower. Again we find numerous goblet cells between the cylindrical cells. They are less numerous in the crypts of the small intestine than in those of the large. Of very frequent occurrence are epithelial cells of the crypts of *Lieberkuehn* in the state of division, this being the case not only in the growing, but also in the mature organism. Their appearance here is so constant that the small intestine of certain animals is selected mainly for the purpose of demonstrating indirect cell division. The left tubule of our specimen shows three nuclei in the state of mitosis, two in the prophase (*loose ball* and *monaster*) and one in the metaphase (*dyaster*). The achromatic spindle can plainly be seen in the latter two. The position of these mitotic figures is also characteristic. While ordinarily the nuclei of the cylindrical cells lie in the lower third of the cell, the mitotic nuclei appear in the upper third; the division is thus preceded by an advance of the nucleus from the cell-base toward the lumen. The mitotic picture is always arranged in a manner that the divisional axis, connecting the two central bodies, is ever parallel to the long axis of the tubule, the ensuing division of the cell-body taking place at right angles to this axis. These mitotic conditions are most often seen in the middle or upper portion of the gland, the base of the gland generally being free from them. Evidently this phenomenon means the substitution of cells, which have been used up. This consumption of cells mainly concerns the goblet cells. The goblet cells, after fulfilling their purpose, disintegrate, and newly formed cells advance from their place of origin.

Besides cylindrical and goblet cells, the crypts of *Lieberkuehn* contain a variety of cells, entirely characteristic to them, the **Panethian cells** (*pz*). They are only found at the base of the gland in form of cells, broader than

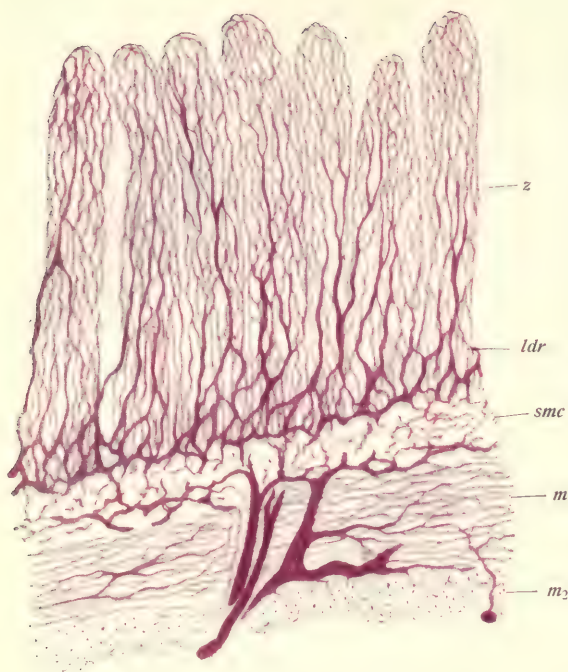


Fig. 127.

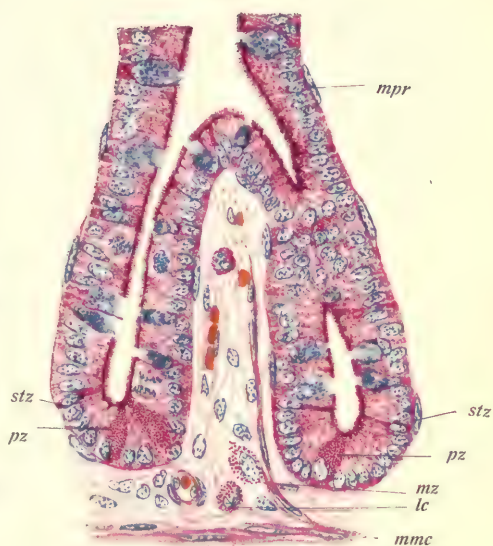


Fig. 126.

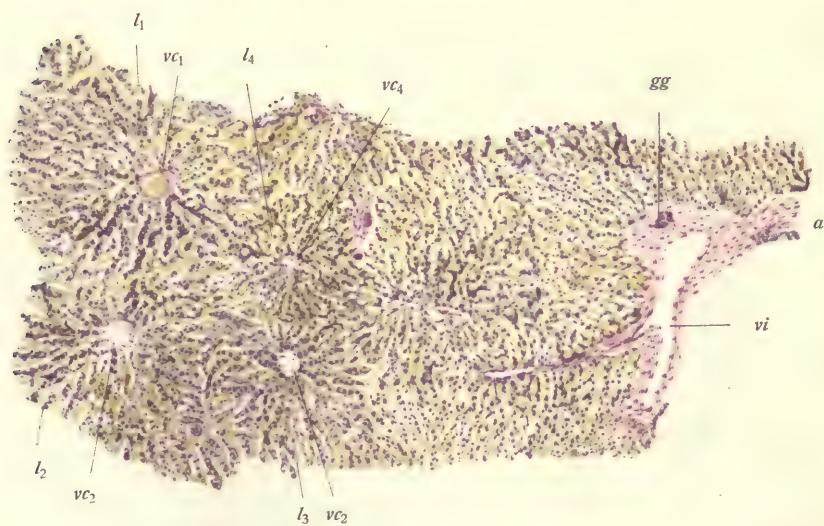


Fig. 128.

the cylindric cells, the bodies of which are filled with medium-sized granules. *Biondi* solution, better than any other staining method, demonstrates just these formations splendidly. If the staining process is followed by rinsing in distilled water instead of dilute acetic acid, the acid fuchsin is strongly extracted from the cellular protoplasm of the cylindric cells; the granules of the *Panethian* cells, however, retain the stain and thus become beautifully differentiated from the pale epithelium. The nucleus is most always found at the cell-base, but may occasionally advance to the cell-centre. In all probability the *Panethian* cells are elements concerned in the preparation of a particular ingredient of the intestinal juice.

Finally we have to mention a last variety of cells, found in the glands of *Lieberkuehn*, narrow, peglike formations (*stz*), which are found in the base of the gland as well as higher up. These cells, always staining extremely dark, are probably disintegrating formations, probably partly *Panethian* cells, which have lost their secretion.

Between the two glands we see the reticular propria; as was the case in the stomach, it contains bundles of smooth muscle fibres, blood-vessels, eosinophilic leucocytes (*lc*) and also a mast-cell (*mz*), which latter are very abundant in the propria in the child. Below, the mucous membrane is bounded by the muscularis mucosæ (*mmc*).

Fig. 127.—Small Intestine of Dog, Showing Injected Blood-Vessels

25. $\frac{3}{4}$. Formalin. Frozen section.

The injection of intestinal vessels is one of the easiest procedures in the technique of injection. In man and the larger animals the cannula may be directly introduced into the superior mesenteric artery or one of its larger branches; in small and medium-sized animals the aorta should be selected at the height of the origin of the renal vessels, a ligature being placed just beneath the origin of the celiac axis. By this method an injection of the entire small intestine is obtained. The intestinal mucous membrane must appear deep red after the injection, the progress of which can very easily be followed. If the mass should be too warm, the muscles of the villi will strongly contract, resulting in a plication of the villi and consequent disturbing tortuosity of the vessels of the villi. After the mass has solidified, the gut is cut open, spread and fixed in 10% formalin. The frozen sections either remain unstained or are weakly stained with a blue protoplasmic dye (*indigocarmin*), in order to have a distinct outline of the villi even with an open diaphragm.

Course of the Blood-Vessels Within the Intestinal Wall.

Our specimen demonstrates how the vessels, coming from the mesentery, pierce the muscular layers in an oblique course, giving off numerous muscular

branches, which surround the muscular fibres in a long, drawn-out reticulum, the individual branches appearing in transverse section in the outer longitudinal layer (m_2) and in longitudinal section in the inner circular stratum (m_1). After reaching the submucosa (*smc*), the arteries form a wide-meshed plexus, from which branches ascend to the propria, where they in turn form a second plexus. The latter supplies capillary nets to the crypts of *Lieberkuehn* and also the arteries of the villi. One to three branches enter each villus, forming a wide-meshed capillary reticulum, the distribution of which we remember from the cross-section of a villus. The capillaries collect in one vein, the further course of which follows the arteries.

Fig. 128.—Human Liver

35. $\frac{3}{4}$. Formalin. Frozen section. Cresyl violet.

The microtechnical preparation of the liver does not present any particular difficulties. For the general study of the human liver, frozen sections of formalin material, stained in cresyl violet (p. 62), will furnish very useful pictures.

Liver-Lobules.

In our specimen the liver-cells are stained blue, the nuclei reddish-violet, the connective tissue pink and the blood bright yellow. Four **liver-lobules** (l_1-l_4) can be distinctly outlined in the left half of our specimen, while toward the right we are unable to distinguish separate lobules. In the centre of each lobule we find a lumen partially (*vc*) filled with blood and surrounded by a scanty amount of connective tissue, the **central vein** (vc_1-vc_4). From this lumen the parenchyma of the lobule radiate in form of cell-beams, which are connected among themselves in net fashion. Between the beams we find the blood-capillaries, filled in this case to their utmost capacity.

Near the periphery, as we notice, the outline of the lobules is extremely incomplete. Only now and then we find between the lobules larger vessels, surrounded by a considerable amount of connective tissue. Our specimen shows a large, empty vessel (*vi*), a branch of which pierces between two neighboring lobules. This is an **interlobular vein**, viz., a branch of the portal system for the following reasons. Close to the vessel a small, transversely cut duct is seen, which, under high power, proves to be lined with cuboid epithelium; this is a small interlobular **bile-duct** (*gg*); a little farther to the right an obliquely cut blood-vessel appears, having a distinct muscular wall (*a*), a branch of the hepatic artery. The branches of the hepatic vein always take an isolated course, never following the tracks of the arteries and bile-ducts, therefore it is evident that we are dealing with a branch of the portal vein.

Interlobular Connective Tissue.

Our specimen illustrates the important fact that, under normal conditions, the interlobular connective tissue of the human liver is very scanty,

This is not always so in animals; the rabbit shows quite a good deal of connective tissue, and in the pig each lobule is surrounded by a complete connective tissue capsule. It is, therefore, recommended to supplement this specimen by one of the pig's liver, the sections being prepared with the same technique.

*Intralobular
Connective Tissue.*

Ordinary staining methods do not show any collagenous tissue in the liver-substance aside from these small amounts of connective tissue. Gold and silver impregnation will demonstrate other elements, namely, a system of intralobular fibres. We can convince ourselves of the presence of these *lattice fibres* very easily by treating our frozen sections with *Gram's* solution-gold method (p. 73). We will find within each lobule a latticework of finer or coarser, at times undulating and tortuous fibres, which surround the blood capillaries and communicate with the interlobular tissue as well as with that surrounding the central veins. Whether or not these are genuine collagenous fibres has not been definitely ascertained.

PLATE 54

Fig. 129.—Liver of the Rabbit

**Fig. 130.—Liver of Rabbit with Injected Blood-Vessels and
Bile-Ducts**

Fig. 129.—Liver of the Rabbit

550. $\frac{3}{4}$. Sublimate-acetic acid. Frozen section. *Biondi* solution.

The liver of the rabbit above all mammals is best fitted to demonstrate the finer structure of liver, and especially the relation between the bile capillaries and the interlobular bile-ducts. Small pieces of the organ are taken from the freshly killed animal and fixed for four to five hours in a 3.5% solution of sublimate which has been acidified with 1% acetic acid; wash in running water overnight and transfer to 5% formalin for twenty-four hours. Very thin sections should be made on the freezing microtome and stained in *Biondi* solution (p. 67).

The Interlobular and Intralobular Biliary Tracts.

We look for a place on the periphery of the lobules where we can find bile-ducts within the interlobular tissue. Our specimen shows a larger bile-duct (gg_5); the lumen is lined by cuboidal epithelium, the cells of which show on their surface a thin cuticular border and distinct intercellular cement pegs. Beneath the epithelium we come to the *membrana propria*, which is externally invested by cells; then we come to the interlobular connective tissue. Close to the bile-duct we find again an obliquely cut interlobular vein (vi), and finally we come to that lobule, on the periphery of which we notice four very minute biliary ducts. Two (gg_1 and gg_2) of these are cut transversely, showing a small stellate lumen, which is surrounded by low cuboid cells. Of greater interest are the two ducts, appearing in oblique section (gg_3 and gg_4). In both we can distinctly observe how the bile-duct lumen is continuous with that of the bile capillaries, the place of the small epithelial cells being taken by the much larger liver-cells.

The Gland Structure of the Liver.

To study the relation of the liver-cells to the biliary capillaries, we select a place where the capillaries are cut transversely. Such is the case at gc ; here we find that the lumen is bounded by two cells. Two prismatic cells are approximated by two surfaces in such a manner that a small canal is left between. In this respect the liver makes an exception to all other glands discussed so far. While we always found the lumen of the secreting end-piece bounded by at least three to four cells, we find here that two cells constitute the rule. Our picture also illustrates the fact that the bile capillaries and blood capillaries never pursue a common course, but are always separated by the substance of the liver-cells.

Another characteristic peculiar to the liver, in contradistinction to other glands, is found in the fact that a liver-cell does not only participate in the formation of one bile capillary, but helps to form several. For instance, a

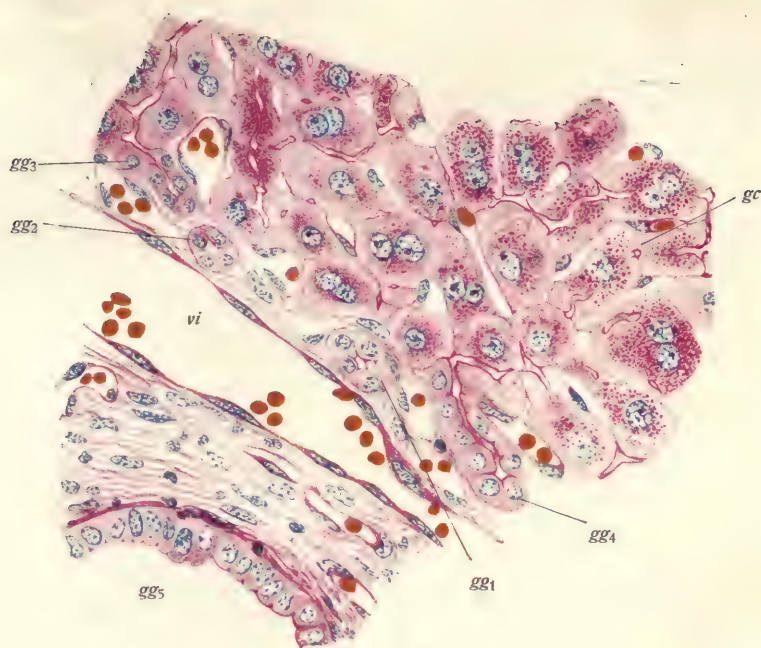


Fig. 129.

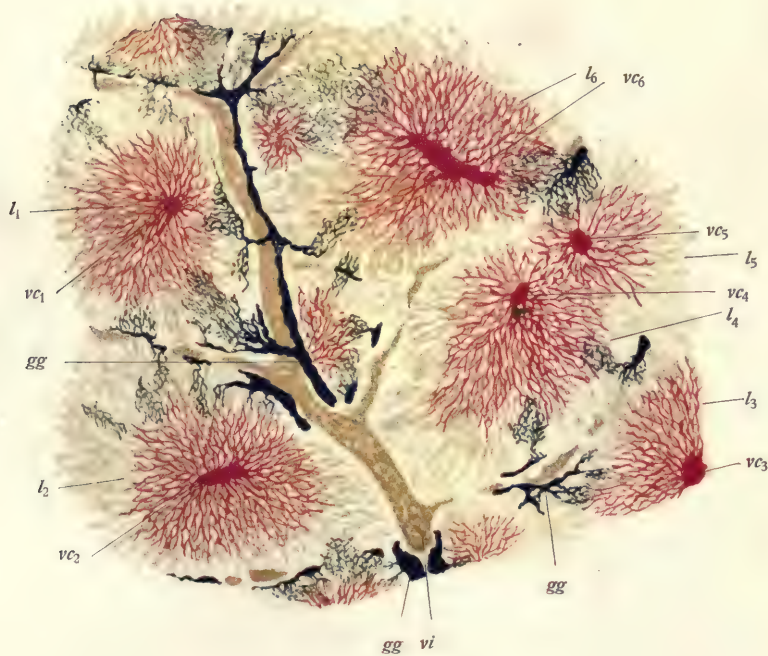


Fig. 130.

cell, rectangular in section, may show a biliary capillary on each side, while each corner is occupied by a blood capillary. Those are places where one cellular beam is in connection with four others. This connection is discontinued after a distance and others are taken up, so that a network of beams is formed, which in turn contains a network of bile capillaries. The liver thus is a tubular gland, the tubules of which branch and form a reticulum. At the periphery of the lobule we find, due to the junction of several neighboring biliary capillaries, numerous small bile-ducts, which again are joined in net-fashion. From the network of smaller ducts, which surround each lobule, arise the larger.

The liver-cells, composing the cellular beams, are of prismatic form, if we combine all the different sectional views which our specimen offers; in mammals they very often contain two nuclei, which came to existence by indirect nuclear division, since mitotic changes are of very frequent occurrence in the liver, especially that of the rabbit. In many cases the two nuclei are differently affected by our staining solution, one staining dark blue and the other very light. Close observation shows that in the former the chromatin has clumped together in thick lumps; we are tempted to consider such nuclei as disintegrating. The nucleus of the liver-cell probably plays an important part in the glandular activity and thereby is used up.

Structure of the Liver-Cell.

The body of the liver-cell, especially from animals which were killed during active digestion, shows a very distinct protoplasmic network, such as we observed in the amphibia (p. II, 4 ff.). During the state of starving, the mesh-work is less distinct. In both cases we find **glycogen** within the meshes, in the first instance in the form of coarse lumps, in the second as fine granules. In our specimen the cells contain abundant masses of bright red glycogen granules. Another constant constituent of the liver-cell is *fat*, which of course is not shown in our specimen, but which can easily be demonstrated by placing a section in 50% alcohol for a few minutes and staining it in an alcoholic solution of sudan (p. 66).

The ectoplasm of the liver-cell during starvation is very thin; after abundant feeding it becomes stronger with the increasing glycogen contents; at those places where the liver-cell bounds the lumen of the bile capillary, it is always well developed, so well, indeed, that we may speak of a wall of the biliary capillary. It is probable that the constant action of the bile has thickened the liver-cell ectoplasm in such places in a similar manner, in which the free surface of the epithelial cells of the bile-ducts appears lined with a cuticle.

Fig. 130.—Liver of Rabbit with Injected Blood-Vessels and Bile-Ducts

35. $\frac{3}{4}$. Formalin. Frozen section.

Since the manner of blood-supply of the liver differs from that of other glands, it will be of advantage to have a closer view of the same. For this

purpose we will precede an injection of the blood-vessels by one of the biliary ducts. The technique of injecting the biliary passages is quite a difficult task. It becomes proportionately harder as the bile of the animal becomes more concentrated and as the interval between death and injection is prolonged. We select a herbivorous animal, the bile of which is much thinner than that of the carnivorous, preferably a very large rabbit. The animal is killed by stabbing through the neck. After opening the abdominal cavity, we search for the stomach. If the pylorus is pulled toward the left side of the animal and we follow along the duodenum, we soon notice, about a finger's breadth below the pylorus, a light yellow duct, coming from the liver, which resists traction of the hand, the ductus choledochus. Lymph and blood vessels share its course. With an artery-hook a double thread is placed around the duct. One-half of the thread serves as a retractor, the other for the tying in of the cannula. The head of the latter should be quite pointed and not more than 2 mm in thickness. The duct is now incised, the empty cannula introduced and tied in. The introduction of the cannula, especially in a small animal, proves quite difficult. Guiding points should be, that the duct is well incised and that the cannula is not tied in, until it is filled with bile, the latter advancing distinctly in the cannula. By gentle massage from without much bile can be delivered. The cannula, free from air, is now connected by a rubber tube to a funnel, containing the filtered solution of Berlin blue (p. 74), the pinchcock is opened, and the injection begins with as little pressure as possible. A syringe may be used, but requires the greatest care, since extravasations are very apt to occur. The injection is discontinued when the mass appears at the surface of the liver.

The subsequent injection of blood-vessels is conducted through the hepatic veins. The superior vena cava is recognized closely above the diaphragm, a ligature is placed and the vessel cut above the ligature; after allowing the blood to flow out, we tie the vessel. The portal vein is also ligated just before its entrance into the liver. A cannula is now tied in the inferior vena cava, below the liver, where it is not as yet surrounded by liver substance. The cannula should be filled with salt solution at 40–50°, and the diluted red mass (p. 74) quickly injected. The injection is incomplete, being discontinued as soon as we notice larger areas of the surface, where the central portion of the lobules appears injected. The mass must not penetrate to the interlobular vessels. After the injection the entire animal is placed on ice, the intestines are pushed aside and the abdominal cavity is filled with ice. It is best to leave the animal there overnight; the following morning the entire organ is cut into moderately sized pieces and fixed for three days in the mixture of *Mueller's* fluid and formalin (p. 34), which must be changed daily. We now wash in running water for twenty-four hours, transfer to 5% formalin, and make thick frozen sections. The best pieces must be selected, as the injection is not by any means equally good in all parts of the organ.

Our specimen shows six liver-lobules (l_1-l_6) in full section, others being only partially sectioned. In the centre of each we find the **central vein** (vc_1-vc_6), partly cut transversely (vc_1 , vc_3 , vc_4 , vc_5), partly in oblique section (vc_2 and vc_6). From the central vein the red injection has travelled more or less to the periphery of the lobule, without transgressing the latter.

The interlobular veirs (*vi*) are not injected, but appear very distinct, being distended with blood. We thus have a natural injection of the afferent and an artificial injection of the efferent vessels.

In company of the interlobular veins we find the ***interlobular bile-ducts*** (*gg*), filled with blue mass. There, too, the injection is incomplete. We notice the branches piercing between the different lobules, whence they give rise to a delicate blue network of bile capillaries, which advances into the lobules for a distance.

PLATE 55

Fig. 131.—Human Pancreas

Fig. 132.—Human Pancreas

Fig. 131.—Human Pancreas

35. $\frac{3}{4}$. Sublimate. Frozen section. Cresyl violet.

The human pancreas is fixed in a 2.5% solution of sublimate for four to five hours, washed in running water overnight, transferred to 5% formalin for twenty-four hours, and cut on the freezing microtome. In staining the sections a principal aim is to differentiate the so-called **islands** of **Langerhans** from the remaining parenchyma; this is best accomplished by staining in cresyl violet (p. 62).

*Division of the Parenchyma
into Lobules.*

Low power will show that the parenchyma of the gland is arranged in small lobules, which are separated by more or less connective tissue. In the interlobular connective tissue we find numerous blood-vessels (*bg*), and furthermore the **interlobular ducts** (*ag*). They are built similarly to the ducts of the salivary glands, but never show the rod-structure of the latter. After entering the lobules they form long intermediate pieces and finally vesicular end-pieces, the alveoli.

Islands of Langerhans. The epithelial cells lining the latter are deep blue in our specimen. From these blue parenchyma of the lobules we can distinctly differentiate masses of cells by their light yellow color; these are the **islands of Langerhans** (*li*). In this case they are very well developed, as many as four to five islands appearing in one lobule at times. They are mostly round, but may be irregular. On close inspection we find the yellow color to be due to the presence of blood. They are crossed by a large amount of relatively wide blood capillaries, the contents of which, viz., the red blood corpuscles, have taken a yellow stain. The cells proper of the islands are stained pale bluish-violet.

Fig. 132.—Human Pancreas

550. $\frac{3}{4}$. Sublimate. Frozen section. *Biondi* solution.

For the study of the structural details we prefer staining a frozen section in *Biondi* solution (p. 67).

*Structure of the Secreting
Alveoli and Their Communication
with the Interlobular Ducts.*

Fig. 132 presents a part of such a section at the junction of the glandular parenchyma (right) and the island of Langerhans (left). The right half, aside from several transverse and oblique sections of alveoli (*a*), also

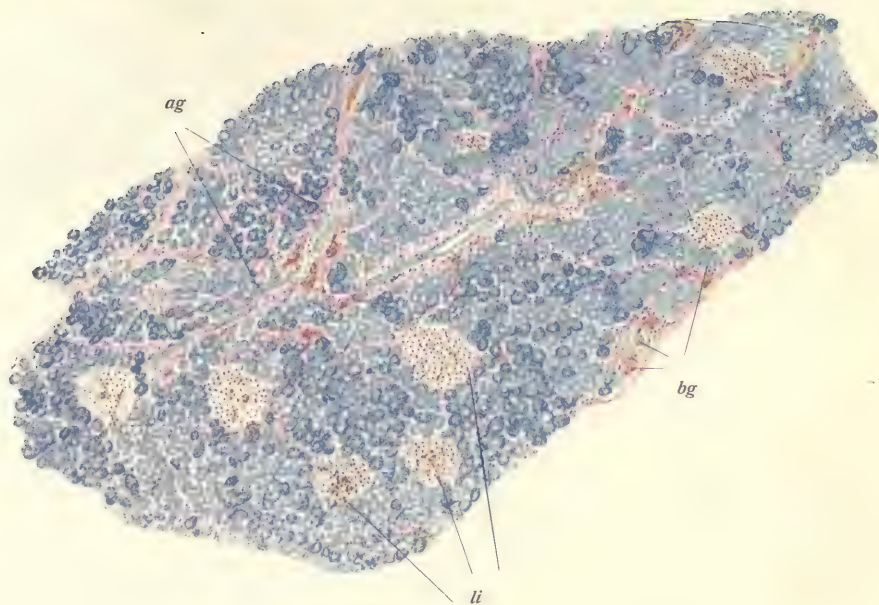


Fig. 131.



Fig. 132.

contains two sections of intermediate or inserted pieces (s_1 and s_2). One of these (s_2) shows the transition into the alveolus. The **intermediate pieces** are narrow canals, lined with low cuboidal cells. The **alveoli** likewise have a narrow lumen. Their shape, as we may compute from the various sections, is ovoid or pyriform; their external measurements exceed by far those of the intermediate pieces, the alveolus on its intermediate piece appearing like a grape on its stem. Where the intermediate piece enters the alveolus, the low epithelia change to the secreting cells of the alveolus. They have in general a cuboid or conical form, showing two distinct zones within their cell-body. The periphery of the cell-body is filled by extraordinarily dense protoplasm, while the central portion shows a distinct light network of protoplasm. The proportionate size of these two zones varies in different alveoli. Sometimes the outer zone is very broad, then again it will be a mere band.

The Living Pancreas Cell.

While in the fixed and stained pancreas cell we find a dark outer and a light inner zone, the reverse is true of the fresh cell. Such a specimen may very easily be obtained from a rabbit. There the pancreas is found as a thin, transparent flat organ between the two folds of mesentery, which connect the two limbs of the horseshoe-shaped duodenal loop. If such a pancreatic lobule, taken from the freshly killed animal, is examined under high power with a drop of *Ringer's* solution, we find that the dark hue of the inner zone is due to the presence of masses of granules, the **zymogen granules**. During secretion they are expelled from the cells and form an important constituent of the secretion. During the ensuing period of rest new granules are formed.

Our specimen will now and then show some granules at the periphery of the section. Most of them, however, have been destroyed by the fixation.

In a similar manner, as noticed on the albumin cells of the salivary glands, we find **secretory capillaries** piercing between the secreting cells.

Furthermore, we find cellular nuclei within the lumen of the alveoli, they belong to the **centro-acinary cells** (cz), constituting a characteristic peculiarity of the pancreas, since they are not found in any other gland of the human body. They are flat cells, lining the alveolar lumen in a very incomplete manner.

Structure of the Islands of Langerhans.

The island of *Langerhans*, occupying the left portion of our specimen, again shows a wealth of blood capillaries. Its parenchyma consists of polygonal, relatively small cells, which have a central nucleus and a light, indistinctly net-form cell-body. Among the light cells we find some darker cells, having smaller nuclei. Perhaps these interesting formations have a secretory nature, the secretion playing in all probability an important rôle in the human economy.

PLATE 56

Fig. 133.—Upper Turbinal of the Guinea-Pig

Fig. 134.—Frontal Section through the Human Larynx

4. THE ORGANS OF RESPIRATION

Fig. 133.—Upper Turbinal of the Guinea-Pig

65. $\frac{3}{4}$. Sublimate. Trichloracetic acid. Paraffin embedding. *Biondi* solution.

In order to gain a general view of the structure of the nasal passages, we select a small mammal, e.g., the guinea-pig. After killing the animal we disarticulate the lower jaw, and, after cutting through the skin, we saw off the entire anterior portion of the head, starting close to the eyes in the frontal plane. The specimen thus obtained is again divided into an anterior and posterior half, by sawing parallel to the first line of section. The posterior half is fixed in 3% sublimate solution for five to six hours. We recommend the use of the air-pump, while the specimen is yet in the fixing solution, since air-bubbles retained in any turbinal meatus will seriously interfere with fixation. After washing the specimen in running water overnight, we suspend it in a larger amount (200–300 cm³) of 5% trichloracetic acid for the purpose of decalcification. The liquid being renewed once, the process will be completed in two to three days. From the acid the specimen is transferred to a 5% solution of *Glauber* salt, where it remains for twenty-four hours, and is then washed for twenty-four hours in running water. Dehydration by the different grades of alcohol is followed by chloroform, and the specimen is finally embedded in paraffin. The sections are made parallel to the original line of section, and after being placed are stained in *Biondi* solution (p. 67).

In the centre of the nasal passage, roofed by the nasal bone and the maxilla, we find the septum nasi; extending from the lateral walls, almost touching the septum, we see the prominence of the turbinals, at least of the upper and middle turbinals.

Structure of the Turbinal.

Making a close inspection of one of these swellings, we find that its foundation is formed by a thin plate of bone (*kn*), which is continuous with the bony lateral wall. Externally we notice an *epithelial* coat, which differs materially on the upper and lower surface respectively. Above we have a very high epithelium (*rep*), comprising a superficial non-nucleated and a deep nucleated zone. In the latter several strata of nuclei are seen. Toward the free edge of the prominence, viz., toward the septum, the epithelium becomes lower and finally (X) merges into a double row of ciliated epithelia (*resp*), which invests the entire lower surface of the protuberance.



Fig. 133.

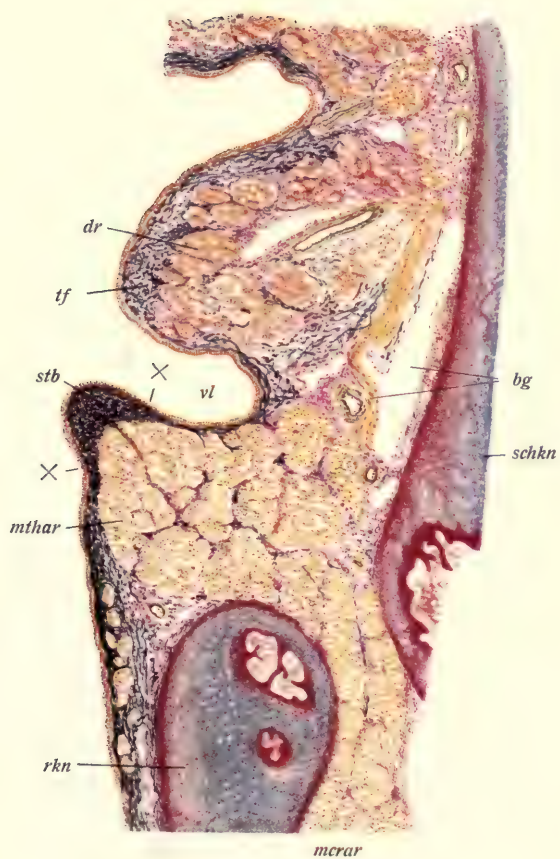


Fig. 134.

*Olfactory and Respiratory
Mucous Membrane.*

The high epithelium of the upper surface is the **olfactory epithelium**. With its structure we will familiarize ourselves, when discussing the organs of special sense (p. II, 374). The double ciliated epithelium, which we have met before, is found in the greater part of the respiratory tract, and hence has been named **respiratory epithelium**.

The respiratory epithelium is mounted on a distinct basal membrane. The latter becomes indistinct, where the olfactory epithelium sets in, and finally disappears entirely. Between the bone and the olfactory epithelium we see the submucosa, containing a mighty glandular stratum (*dr*). They are the **olfactory** or **Bowman's glands**, branching tubular glands, which open into a small sinus, situated beneath the olfactory epithelium, which opens between the olfactory cells (*drg*). They are purely serous glands, furnishing moisture to the nasal mucous membrane.

The respiratory mucous membrane also contains glands, but of a different form and smaller calibre. They are less numerous in the guinea-pig than in man. Our picture does not show them. They are similar to the glands which we will meet in the deeper parts of the respiratory apparatus.

There is an abundant supply of blood-vessels in the submucosa of the nasal mucous membrane. In the regio olfactoria we find between the glands numerous longitudinal and transverse sections of non-medullated nerve-fibres; they are branches of the olfactory nerve.

Fig. 134.—Frontal Section through the Human Larynx

15. $\frac{3}{4}$. Sublimate. Nitric acid. Frozen section. Paracarmin. Resorcin fuchsin, picrofuchsin.

Frontal sections furnish the best demonstration of the microscopical structure of the most important parts of the human larynx. The larynx is split into a right and left half by a median incision, fixed in a 3% sublimate solution for five to six hours, and washed until the following day. Since the laryngeal cartilages calcify at the age of twenty (a little earlier in the male than in the female), such specimens must be decalcified. This end can be achieved by suspending them for twenty-four hours in a 5% solution of nitric acid. To remove the acid, we place the specimen in a 5% solution of *Glauber* salt for twenty-four hours. After washing in running water for twenty-four hours we can transfer to 5% formalin for a few days. Frozen sections are made of a piece, 3–5 mm in width, its two longitudinal surfaces corresponding to two cuts, made at right angles to the vocal band. The sections, which should not be too thin, are placed in 70% alcohol, stained in paracarmin (p. 55) for fifteen minutes, and washed well in 70% alcohol. We counter-stain first with resorcin fuchsin (p. 63) for fifteen minutes. After extracting the excess dye by 95% alcohol, changed repeatedly, we transfer the sec-

tions to picrofuchsin (p. 67) for five minutes. After rinsing in 70% alcohol and dehydrating we mount in Canada balsam.

Fig. 134 shows a section prepared in this way. At the right we recognize the cross-sections of the two largest laryngeal cartilages, the **thyroid cartilage** above (*schkn*) and the **cricoid** below (*rkn*). Their basic substance has been colored grayish-violet by the resorcin fuchsin. Ossification has already taken place in the cartilages. In the lower edge of the thyroid as well as in the upper border of the cricoid cartilage we can see a small narrow canal, filled with bone marrow, small lamellæ of bone projecting into the canal.

Structure of the Vocal Band.

Extending from below, between the ends of the two cartilages, we see a large mass of transversely cut striated muscle, which protrudes inward and upward in a bulky swelling. This mass of muscle comprises the crico-arytænoideus lateralis muscle (*mcrar*) below and the thyreo-arytænoideus (*mthar*) above, which at this place cannot even be differentiated by the microscope. The latter has again been subdivided into an inner portion, the vocal muscle, and an outer portion, the thyreo-arytænoideus externus. The protuberance, formed in the larynx by the vocal muscle, is completed by the **vocal band** (*stb*), mounted thereon in a median position. It is composed, as we can readily see in our specimen, of transversely cut, quite thick elastic fibres, thus forming an elastic cord.

Above the thyreo-arytænoideus externus we find between thyroid cartilage and laryngeal lumen not only large blood-vessels (*bg*) and scanty ascending muscular bundles (thyreo-arytænoideus superior), but also loose connective tissue, containing numerous masses of glands. These cause the laryngeal mucous membrane to project a fold into the lumen of the larynx, known as the **pocketfold** or the **false vocal bands** (*tf*). The latter does not extend as far into the lumen as the true vocal bands. In more anterior sections we can see the appendix emanating from the laryngeal ventricle (*vl*), projecting far upward.

Laryngeal Mucous Membrane.

Examining the mucous membrane of the larynx we find it to be composed of **double ciliated epithelium**, such as we have met in the respiratory part of the nasal passages. One place, however, always makes an exception, and that is the free portion of the vocal band projecting into the lumen of the larynx. Here ($\times - \times$) the ciliated epithelium is replaced by **stratified flat epithelium**. The latter form may also be seen in other places, e.g., on the surface opposite the false cords; likewise we find the entire laryngeal surface of the epiglottis coated with a more or less broad border of stratified flat epithelium.

The epithelium is mounted on a **basal membrane**, which under high power presents a bright, yellowish red color. Below it is the *propria*, which contains numerous elastic fibres, the latter becoming denser farther inward, so that, e.g., in the false vocal bands, we may speak of a separate elastic

band, the ventricular ligament. In the region of the labium vocale the epithelium lies directly upon the vocal ligament, and here we find rugæ in the propria, not very distinctly shown in our picture, which run longitudinally in the labium vocale, the cross-section showing them as papillæ, projecting into the epithelium.

Glands are abundant in the laryngeal mucous membrane, most numerous in the pocketfold or false bands. The region of the true vocal bands is absolutely free from them.

PLATE 57

Fig. 135.—Transverse Section through the Trachea of a Child

Fig. 136.—Lung of Calf

Fig. 135.—Transverse Section through the Trachea of a Child

60. $\frac{3}{4}$. Formalin. Frozen section. *Biondi* solution.

Small circular pieces of the trachea of a child are fixed for twenty-four hours in 10% formalin, followed by 5% formalin for an equal length of time. The frozen sections are stained in *Biondi* solution (p. 67). The staining method employed in the previous specimen also furnishes very good results, especially for the demonstration of the great development of elastic tissue.

Structure of the Tracheal Wall.

In general the tracheal wall presents similar conditions as seen in the laryngeal wall. Low power shows especially the deep blue **tracheal cartilage** (*kn*), which, as we know, has the shape of a ring, open at the posterior aspect. The ring is completed by strong bundles of smooth muscle, running from one end of the cartilage to the other, thus forming the main constituent of this membranous part (*pars membranacea*) of the trachea. The cartilaginous crescents consist of hyaline cartilage, which is surrounded by a coarse perichondrium.

Inspecting the tracheal mucous membrane with higher power, we recognize at once the double layer of **ciliated epithelium** (*ep*) forming the surface. It contains numerous goblet cells, which are easily recognized in our specimen by their blue color. The epithelium rests on a quite distinct **basal membrane**. Below the latter we find a narrow stratum of longitudinal connective tissue fibres, containing leucocytes, which does not appear in our picture, followed by a layer of longitudinal **elastic fibres** (*ef*) of considerable size. In the *pars cartilaginea* it is generally well defined from the remaining *propria*, while in the *pars membranacea* the *propria* is evenly blended with elastic fibres. A tangential section shows that the fibres form a narrow-meshed network.

That portion of the **propria** (*pr*) situated between this subepithelial, elastic stratum and the perichondrium contains, aside from the connective tissue basic substance, elastic fibres, blood-vessels and nerves, also numerous **glands** (*dr*). They are found principally in the middle and deep strata of the *propria*, but may be seen higher, beneath the epithelium. They are most numerous in the *pars membranacea*, occurring as far outward as between the muscle fibres. They are typical *mucous glands*, their cells being filled with blue secretory granules. The duct (*drag*) generally crosses the *propria* in an oblique course, forming a slight sinus before arriving at the epithelium, and finally opens, its cells merging into the ciliated surface epithelium. It is lined by simple or double cylindric epithelium. Between the cylindric cells numerous goblet cells are found. At times we find cilia upon the cylindric cells of the aforementioned sinus of the duct.

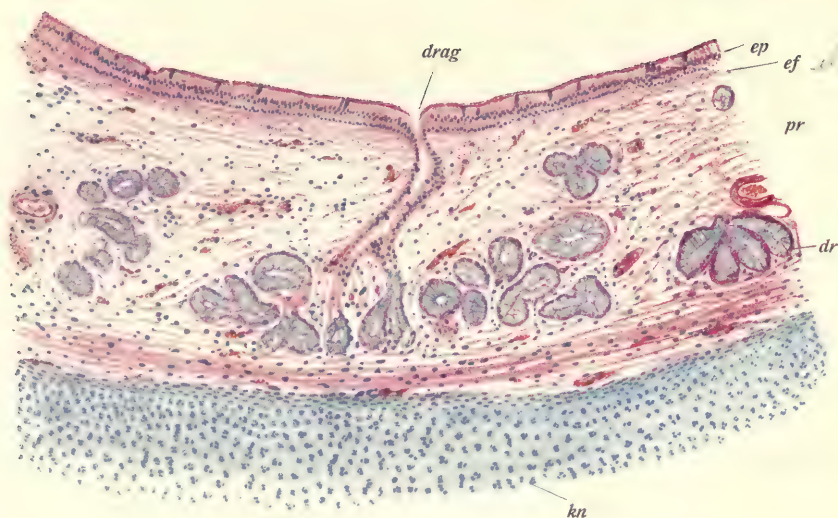


Fig. 135.

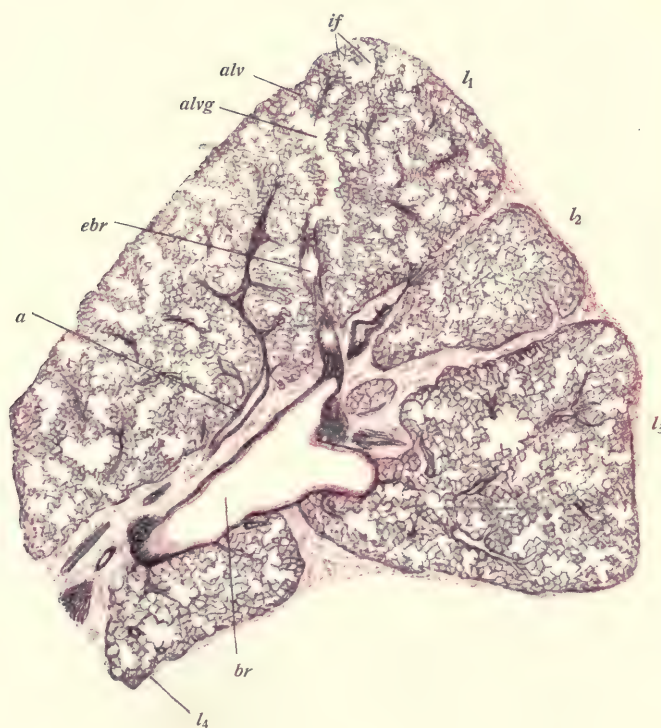


Fig. 136.

Fig. 136.—Lung of Calf

20. $\frac{3}{4}$. Formalin. Frozen section. Paracarmin. Resorcin fuchsin.

It is quite difficult to present to the beginner a specimen showing the general gross-structure of the lung. It necessitates thick sections, 25–50 μ , which, of course, must be stained correspondingly. It is quite impossible for the beginner to become acquainted with the structure in a thin specimen. The lung of a grown person is ill-suited for the purpose, since the large size of the lobules interfere with a general view. Much smaller and hence better for demonstration are the lobules of the infantile lung; however, we prefer to use the lung of the calf, which presents the lung structure in almost diagrammatic form, without differing in any of its essentials from the human lung.

It is absolutely necessary to inject the fresh lung with the fixing solution through the trachea. A suitable lobe is selected, separated from the rest of the organ, and a glass cannula, provided with tube and funnel, is tied into the respective bronchus. Five per cent. formalin is allowed to run slowly into the lobe, until the latter has expanded to its maximum capacity, when the pinchcock is closed and the entire lobe placed in the same solution. After two days the specimen will be hard enough to allow suitable pieces being cut out, of which sections are made on the freezing microtome. The thickness should be 25–50 μ or more. The sections are transferred to 70% alcohol, stained in paracarmin (p. 55), and counterstained in resorcin fuchsin (p. 63).

The Interlobular Branches of the Bronchi.

First we inspect the large bronchial branches with their accompanying branches of the pulmonary artery; here we find that the cartilage decreases in amount with the decrease in calibre of the bronchus, the cartilaginous plates becoming smaller and smaller and finally disappearing; this change is accompanied by a reduction in glands.

The Pulmonary Lobules and the Intralobular Branching of the Bronchioles.

Following the course of a bronchus, we finally come to places such as depicted in Fig. 136. Here we see four different lobules (l_1 – l_4) in section. They are separated by fairly strong connective tissue septa, and between them we find, in oblique section, a **bronchiolus** (*br*), the wall of which is composed of a layer of longitudinal elastic fibres, lined by a low, ciliated, cylindrical epithelium. To the left of the bronchiolus we find the tortuous, accompanying artery (*a*), cut at several places. The bronchiolus divides into branches, one of them being shown here in its entire length, which enter the neighboring lobules. This intralobular portion of the bronchiole is termed the **end bronchiolus** (*ebr*). The latter gives off several side branches (two appear in our picture, cut off), and then breaks up into a number of **alveolar ducts** (*alvg*). These have a more or less larger calibre than the

end bronchiolus, and are studded with numerous air-vesicles, the **alveoli** (*alv*), which open into their lumen. The alveolar duct again dividēs into several blind **infundibula** (*if*), which are also studded with alveoli.

The artery (*a*) enters the lobule with the bronchiolus and then divides into numerous branches, which form a capillary basket around the alveoli. These baskets can only be recognized in specimens which have been injected with glue-mass.

PLATE 58

Fig. 137.—Lung of Calf

Fig. 138.—Human Kidney

Fig. 137.—Lung of Calf

80. $\frac{3}{4}$. Formalin. Frozen section. Paracarmin. Resorcin fuchsin.

*Structure of the End
Bronchiolus, the Alveolar
Duct and the Alveoli.*

To make further studies of our specimen we will use a higher power. We are especially interested in the junction of the end bronchiolus and the alveolar duct. Fig. 137 illustrates this point. The wall of the **end bronchiolus** (*ebr*) consists of a tapering layer of elastic fibrils, lined on the inside by a simple, low, cuboid epithelium. The wall of this end bronchiole is already studded with **alveoli** (*alv₁*) to some extent. In the beginning they are few in number, but become more numerous; the end bronchiole merges into the wide **alveolar duct** (*alvg*), into which the alveoli (*alv₂*) open everywhere. We notice how the elastic tissue of the end bronchiolus extends into the alveolar wall, weaving a net of fine elastic fibres around the *alveoli*, thus forming the main constituent of the alveolar wall.

If we desire to make a more detailed study of the latter, we must stain some sections in *Biondi* solution (p. 67). We will then be able to recognize fairly large, flat cells, lining the alveoli, the **respiratory epithelium**, which is the direct continuation of the cuboid epithelium of the end bronchioles. Externally to these cells we find the elastic tissue and the capillaries, embedded in a structureless basal membrane. The capillaries disappear almost entirely in the specimens injected in this manner.

5. THE URINARY AND GENITAL ORGANS

Fig. 138.—Human Kidney

15. Formalin. Frozen section. Cresyl violet.

Kidneys of children during the first and second years of life are best suited. A median longitudinal section divides the organ into an anterior and posterior half. Each of these is divided by a horizontal cross-section into an upper and lower quarter. The pieces are fixed in 10% formalin for twenty-four hours, followed by 5% formalin for an equal length of time. Plates of 2–3 mm thickness are cut and frozen sections, 10–20 μ in thickness, are stained with cresyl violet (p. 62).

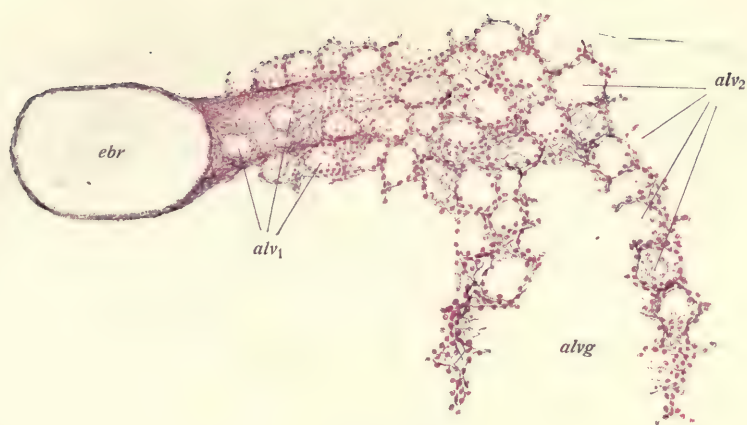


Fig. 137.

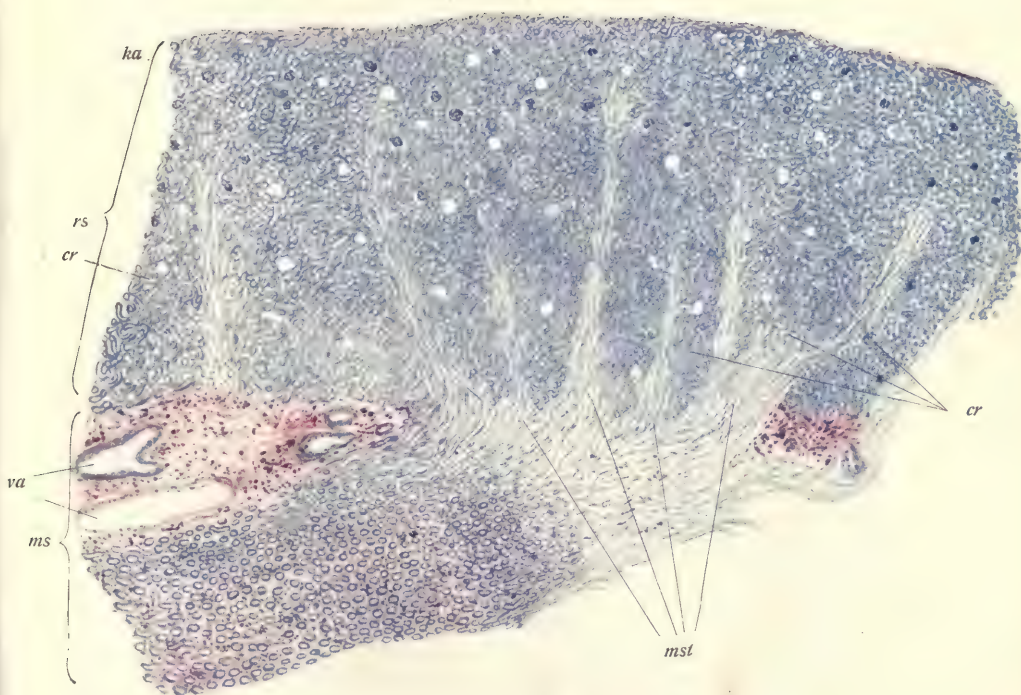


Fig. 138.

*Distribution of Cortical
and Medullary Substance.*

The macroscopic division of the kidney into **cortical** and **medullary substance** (*rs* and *ms*) is likewise noted under the microscope with low power. Internally to the connective tissue capsule (*ka*) we find the uniform, narrow cortex, which soon divides into the **columnæ renales** (*cr*), arising by broader or narrower bases. They are conical or cylindrical divisions of the cortical substance, which taper toward the medullary substance. Where they merge into the latter we find large vessels, surrounded by connective tissue, which run parallel to the surface of the kidney, the **vasa acriformia**.

The medullary portion of the human kidney forms the macroscopically visible **pyramids**, eight to eighteen in number, the rounded apices of which point toward the hilum of the kidney. The broad bases of the pyramids merge, thus forming a uniform medullary substance, from which finer or coarser processes, the **medullary rays** (*mst*), radiate toward the cortex, separating the different columnæ renales.

*Glandular Structure
of the Kidney.*

The kidney is an alveola-tubular gland, the secretory part of which has its origin in a small vesicle, the **Malpighian corpuscle**. We can see this formation represented in large numbers in the cortex; they are wanting in the outermost portion of the cortex, but in the columnæ renales they are found penetrating deeply toward the medulla. We notice in our specimen that the corpuscles themselves have frequently fallen out, a small circular vacuole appearing in their place. From the corpuscle develops a small canal, arranged in many convolutions, the **tubulus contortus** (convoluted tubule). It enters a medullary ray, descending as a thin tubule in a straight course into the medullary substance. Turning abruptly, it retraces its course upward to the vicinity of its point of exit in the cortex. Thus a more or less extensive loop has been formed, termed **Henle's loop**. After arriving near its corpuscle, the tubule again becomes convoluted within the cortical substance and then merges into the *intermediate piece*. This again enters a medullary ray and becomes the **collecting tubule**. As we advance toward the medulla, we find more and more intermediate pieces opening into the collecting tubule within the medullary ray. The small collecting tubules become larger collecting tubules, which finally unite to form the ductus papillares in the deeper strata of the medulla, opening in ten to twenty-four orifices at the papillæ in the hilum of the kidney.

Our specimen does not show the entire course of the tubule. In the deeper and middle layer of the medulla the collecting tubules are mostly cut transversely, surrounded by a small amount of connective tissue. At the basis of the pyramids and furthermore within the medullary rays they are in longitudinal section.

A renal tubule in its entire length, from *Malpighian* corpuscle to the ductus papillaris, can only be represented by way of plastic reconstruction. Considerable parts of it, however, can be obtained by macerating half of a mouse

kidney in hydrochloric acid (*acidum hydrochloricum concentratum*, Pharmacopœia). The kidney is left therein overnight and then transferred for one to two days in repeatedly changed water. Such a specimen is spread on a slide in diluted glycerine. We will be able to find, e.g., the ductus papillares with their respective collecting tubules, *Henle's* loops, *Malpighian* corpuscles with the origin of the tubulus contortus, etc.

PLATE 59

Fig. 139.—From the Cortical Substance of the Human Kidney

Fig. 140.—From a Medullary Ray of the Human Kidney

Fig. 141.—From the Medullary Substance of the Human Kidney

Fig. 139.—From the Cortical Substance of the Human Kidney

280. $\frac{3}{4}$. Formalin. Frozen section. Cresyl violet.

We will now examine some characteristic parts of the preceding specimen under higher power. First we search the cortical substance for *Malpighian* corpuscles, in which the origin of the tubulus contortus has been preserved. With due patience we will succeed.

Structure of the Malpighian Corpuscles.

In Fig. 139 we find a globular *Malpighian* corpuscle surrounded by a **membrana propria** (*mp*). The latter is a structureless membrane, which is continuous on the one hand with the adventitia of the afferent blood-vessels, on the other with the membrana propria of the tubulus contortus. Few connective tissue cells are found on its exterior. Internally to membrana propria we see a stratum of flat, polygonal cells (*pb*). They increase in height at the point of origin of the **tubulus contortus** (*tc*), gradually becoming continuous in the cuboid cells of the latter. On those places of our section, where the contents of the *Malpighian* corpuscle have fallen out, the wall is formed solely by the membrana propria and this simple layer of epithelium. Both together form the so-called *Mueller's* capsule of the corpuscle.

Inspecting the capsular epithelium at the point of entrance of the vessels into the corpuscle (*e*), we easily recognize how here (\times) the capsular epithelium is reflected over the glomerulus, continuing as a layer of low cells, which cover the glomerulus completely (*vb*). We have, therefore, an intimate connection between the glomerulus and *Mueller's* capsule. Due to the fact that this part is but a thin stem, enveloping the blood-vessels, each part of our frozen section which does not cross this spot will be minus the corpuscle, which has fallen out, since its connection with the capsule has been severed.

The **glomerulus** itself, as we soon will observe, is formed by a tuft of capillary convolutions, which are enclosed by the aforementioned epithelium. The vessels, piercing into the corpuscle, originally nothing but a vesicle lined by epithelium, have pushed this epithelium before them, so that the globular vesicle has been transformed into a double-walled goblet. The outer wall of the goblet is formed by *Mueller's* capsule, the inner wall by the reflected epithelium, the edge of the goblet is represented by the place of entrance of the vessels, its contents by the tuft of vessels, and the foot by the tubulus contortus. Between the two walls of the goblet is a small space, the remainder of the former vesicular lumen, which opens into the tubulus contortus. The epithelium, covering the tuft, can in the adult person not be spoken of as



Fig. 139.

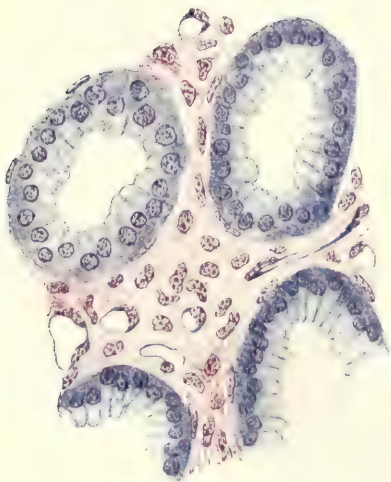


Fig. 141.



Fig. 140.

consisting of separate cells, the nuclei lying in a common, homogeneous mass of protoplasm. Thus we have here a **syncytium**.

A small artery, entering the glomerulus, the **vas afferens** (*va*), can be traced to the left to its origin from the interlobular artery (*ai*). The **vas efferens**, emerging from the glomerulus close to the vas afferens, has not been included in our section.

As regards the **tubuli contorti**, the convoluted uriniferous tubules, we have seen their cuboid ciliated epithelium previously in the frog (p. II, 27). In man and the mammalia they are characterized by a distinct rod-structure at the basal part of the cell, such as we have noticed in the salivary tubules of the submaxillary gland.

To the left of the *Malpighian* corpuscle we observe an **intermediate piece** (*scha*) in longitudinal section. In general it has the same structure as the convoluted tubule; the cells, however, are slightly lower.

Fig. 140.—From a Medullary Ray of the Human Kidney

280. $\frac{3}{4}$. Formalin. Frozen section. Cresyl violet.

Structure of the Straight Uriniferous Tubules.

We now inspect a medullary ray. If what we have previously said be true, we must find **Henle's loop** and **collecting tubules**. Longitudinal sections are most easily recognized, showing the thin limbs of **Henle's loop** (*hschl*¹), quite narrow canals, lined by very low cells. Following their course, we come to a point where the thin limb becomes considerably thicker (*hschl*²), the lumen becoming wider and being lined with higher cuboid cells. This change may occur in the descending as well as in the ascending portion of the loop, so that the caliber of the tubule may increase either in the direction of the medulla or that of the cortex. Quite often we can notice the rod structure in the cells of these thickened portions of **Henle's loop**.

Our section also demonstrates a collecting tubule (*sr*). The moderately wide lumen is lined with cuboid cells.

Fig. 141.—From the Medullary Substance of the Human Kidney

280. $\frac{3}{4}$. Formalin. Frozen section. Cresyl violet.

Structure of the Papillary Ducts.

Finally, we will glance over the large ductus papillares of the medullary substance. They are lined with cylindrical cells, which are of a striking character. Their heads, projecting into the lumen, are very light, appearing al-

most empty, while the deeper part, harboring the nucleus, contains a dark-staining, dense protoplasm.

*The Connective Tissue
of the Kidney.*

While in the cortex we find but scanty connective tissue, wound about the *Malpighian* corpuscles and the convoluted tubules and accompanying the vessels to a large extent, it becomes quite noticeable in the medullary substance, separating the papillary ducts within the papillæ in considerable amounts.

PLATE 60

Fig. 142.—Injected Human Kidney

Fig. 143.—Glomerulus from an Injected Human Kidney

Fig. 144.—Transverse Section through the Human Ureter

Fig. 142.—Injected Human Kidney

20. *Mueller's fluid. Formalin. Frozen section.*

One of the easiest problems in the technique of injection is the injection of the arteries of the kidney. The organ should be removed from the body, placed in warm water (40°) for several hours, and then injected with the red glue-mass (p. 74) by introducing the cannula into the renal artery. The best results are obtained from incomplete injections. Injection should be discontinued when the capsule commences to be red. Complete injections do not yield clear pictures.

Course of Blood-Vessels in the Kidney.

In our previous general view we noticed the curved **arciform arteries**, running parallel to the surface of the kidney. We see one in this specimen filled with red mass (*aa*). From it a strong branch arises at right angles, going toward the cortex and giving off numerous branches in its course, the **interlobular artery** (*ai*). The trunk of the latter as well as its branches constantly give off short side-branches, which as **vasa afferentia** pierce each a *Mueller's* capsule, where they form a **glomerulus**. The vas efferens emerging from the latter breaks up into capillary meshes, which surround the convoluted tubules. The last end branches of the interlobular arteries also form capillaries, which surround the tubules, situated in the outermost portion of the cortex. The capillaries, enclosing the straight tubules of the medullary substance, are partly derived from the vasa efferentia (*ar*₁), partly directly from the arciform artery (*ar*₂).

Where the mass has penetrated into the veins, we find closely under the capsule the stellate veins, **venae stellatae**, which collect the blood from the external part of the cortex and empty into the **interlobular veins** or the **arciform veins**, respectively, the latter following the course of their corresponding arteries.

Fig. 143.—Glomerulus from an Injected Human Kidney

280. *Mueller's fluid. Formalin. Frozen section.*

The Vascular Tufts.

Bringing a single glomerulus in the field under high power, we notice how the **vas afferens** (*va*), after piercing into the *Malpighian* corpuscle, breaks up into numerous anastomosing loops. By the junction of the loops

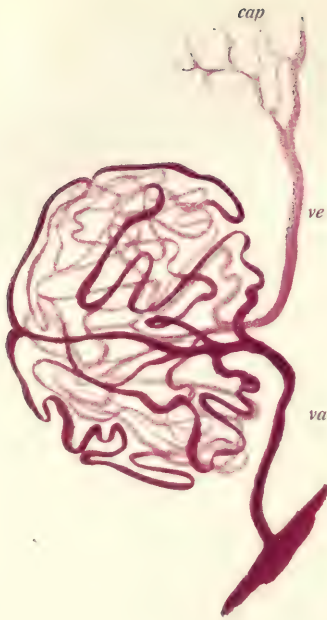


Fig. 143.



Fig. 142.

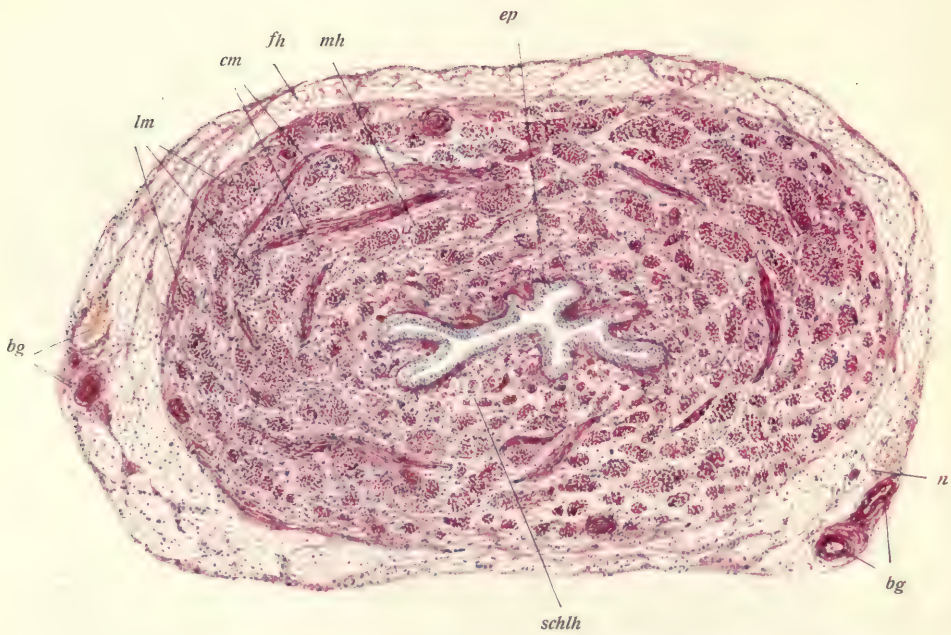


Fig. 144.

a second vessel is formed, the *vas efferens* (*ve*), which, after leaving the corpuscle, divides into capillary loops, surrounding the uriniferous tubules. The entire mass of loops often forms incomplete, separate lobules.

As mentioned previously, the *vas afferens* as well as the *vas efferens* must be considered as arteries, according to the structure of their walls, containing distinct circular muscle fibres. The loops of the glomerulus do not contain any muscle; furthermore, silver injection shows that their endothelium is not composed of distinctly separate cells.

Fig. 144.—Transverse Section through the Human Ureter

30. $\frac{3}{4}$. Formalin. Frozen section. *Biondi* solution.

Small pieces of the organ are fixed in 10% formalin, transferred the following day to 5% formalin, and cut into transverse sections on the freezing microtome. Staining in *Biondi* solution (p. 67).

Structure of the Ureter.

Externally the organ is enveloped by the ***fibrous membrane*** (*fh*), which contains numerous small blood-vessels (*bg*) and nerves (*n*), together with a large amount of fat-cells. This is followed inwardly by a powerful ***muscular coat*** (*mh*). It consists of smooth muscle fibres, which, joined in smaller or larger bundles, partly follow a circular (*cm*), partly a longitudinal (*lm*) course. Although the bundles mingle to a large extent, still we may recognize three different layers. Externally, underneath the fibrous coat, and internally, near the mucous membrane, their course is principally longitudinal, the circular fibres lying between these two strata. The muscular bundles are of varying thickness, separated by abundant connective tissue. Interiorly the longitudinal bundles become steadily thinner, the muscular coat merging into the thin *mucous membrane* without any definite border. The submucosa is wanting.

The mucous membrane (*schlh*) forms several folds, projecting into the lumen. It does not contain any glands and is coated by ***transitional epithelium*** (*ep*), such as we have found in the urinary bladder (p. II, 47).

PLATE 61

Fig. 145.—Urinary Bladder of the Child

**Fig. 146.—Longitudinal Section through the Testicle of the
Monkey**

Fig. 145.—Urinary Bladder of the Child

30. $\frac{3}{4}$. Formalin. Frozen section. *Biondi* solution.

The bladder is removed from the fresh body of a child, split open longitudinally and spread on a wax plate, external surface down. After fixing the specimen for twenty-four hours in 10% formalin, it is cut into strips, 0.5–1 cm thick, vertical to the surface of the mucous membrane, cut and preserved in 5% formalin. Sections are made on the freezing microtome and stained in *Biondi* solution (p. 67).

Structure of the Urinary Bladder.

The bladder-wall in general assimilates that of the ureter. The **epithelium** (*ep*) is the same as that of the ureter; its structure and varying thickness, dependent upon the contraction or distention of the bladder respectively, has been sufficiently discussed in a previous chapter (p. II, 46). The mucous membrane is absolutely free from glands. The **propria** (*pr*) is connected with the muscularis by an indistinct **submucosa** (*smc*).

The smooth muscle bundles, composing the muscular coat, are thicker on an average than in the ureter and are separated by an abundant amount of connective tissue. They cross in all directions, hence appear in transverse, longitudinal and oblique section.

Fig. 146.—Longitudinal Section through the Testicle of the Monkey

6. $\frac{3}{4}$. Formalin. Frozen section. Cresyl violet.

For the study of the gross structure of the testicle we have selected the testis of the monkey, which assimilates the human testis in all essentials. After removing the coverings, the organ is fixed *in toto* in 10% formalin, then split in halves by a sagittal, longitudinal section, and transferred to 5% formalin. Frozen sections of moderate thickness are stained in cresyl violet (p. 62).

Connective Tissue of the Testicle.

The testicle is surrounded by a strong connective tissue capsule (*alb*), containing numerous blood-vessels (*bg*); it comprises the visceral lamina of both the **tunica vaginalis propria** and **tunica albuginea testis**. It sends numerous connective tissue **septa** into the organ, which in a radiat-

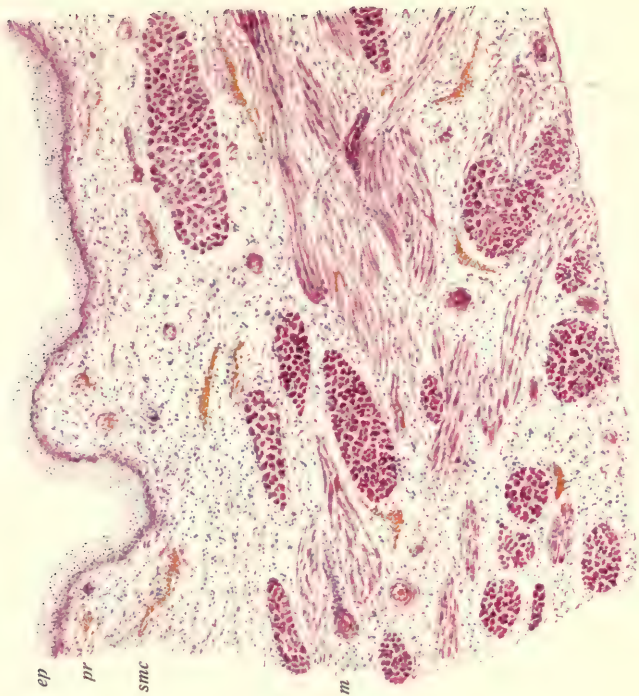


Fig. 145.

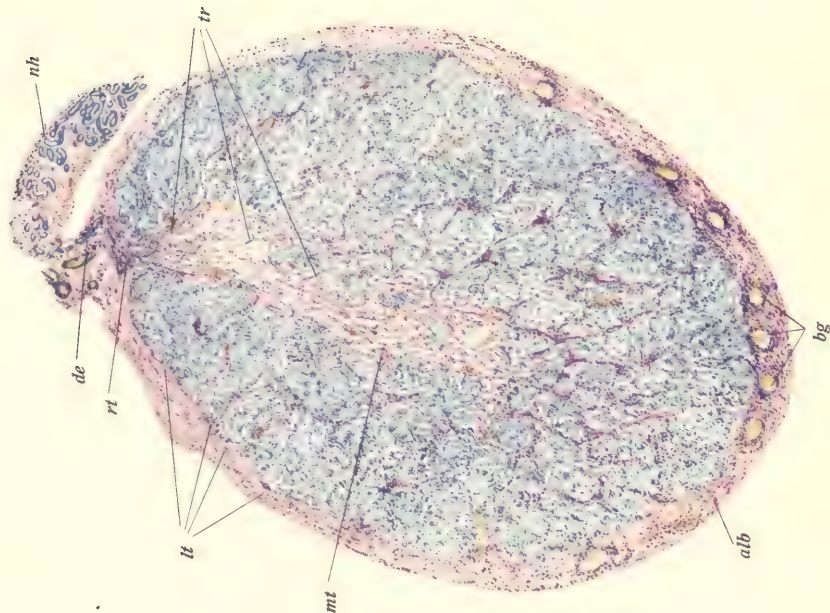


Fig. 146.

ing course reach a mass of connective tissue (*mt*) in the interior of the testicle, the **mediastinum testis**. The latter opens at the postero-superior periphery of the testicle, where it becomes continuous with the albuginea.

*The Parenchyma
of the Testicle.*

The parenchyma is situated within these conical spaces, which envelop the septula testis, forming cone-shaped lobules, the *lobuli testis*, their base being directed toward the periphery, their apex toward the mediastinum. In our specimen they are shown mostly in transverse or oblique section. In the upper part only do we see a few longitudinal sections (*lt*).

Of the efferent ducts of the testicle, the **ductuli efferentes** (*de*), only one is shown here. They are derived from the **rete testis** (*rt*), a fine network, occupying the upper posterior pole of the testis. The mediastinum appears crossed by fine blue canaliculi (*tr*), which empty into the rete testis, the network of the latter being formed by their junction. Following these **tubuli seminiferi testis** toward the periphery, we see them enter the testicular lobules. Thus the straight seminiferous tubules are the efferent ducts of the testicular lobules.

Looking at the apex of a testicular lobule with high power, we readily observe how the tubulus rectus divides, its branches arranging themselves in numerous contortions; the tubuli seminiferi recti merge into the **tubuli seminiferi contorti**. They form the bulk of the lobules, anastomosing among themselves or ending blindly; they represent the semen-secreting portion of the testicular parenchyma. The spermatozoa, as we will soon convince ourselves, are but modified derivatives of the epithelial cells lining these contorted seminiferous tubules.

The efferent ducts enter the epididymis (*nh*) and, again becoming tortuous, they form several small lobules, separated by connective tissue, which combine to form the head of the epididymis, **caput epididymidis**. A duct, **ductulus epididymidis**, emerges from each of these lobules, all of them combining to form the **duct of the epididymis, ductus epididymidis**.

PLATE 62

Fig. 147.—Testicle of the Cat

Fig. 148.—Epididymis of Man

Fig. 147.—Testicle of the Cat

400. Sublimate. *Mueller's* fluid-acetic acid. Paraffin section. *Biondi* solution.

Functionating, i.e., semen-secreting human testes being difficult to obtain, we will study the finer structures of the testicle in a mammal. We select the testes of the cat. The organ is delivered from its coverings and *in toto* placed in a mixture of sublimate, *Mueller's* fluid and acetic acid (p. 31), heated to body temperature. After half an hour the organ is divided into pieces, several millimetres in thickness, which are replaced in the fluid for six to eight hours. After washing them in running water overnight, we dehydrate in alcohol and embed in paraffin. The sections are placed and stained in *Biondi* solution (p. 67). The other testicle is placed in 10% formalin for twenty-four hours and divided into smaller pieces, which are kept in 5% formalin. Frozen sections, made of this material, are first stained in hæmalum (p. 56) for ten minutes, washed in water, transferred to 50% alcohol and counterstained in an alcoholic solution of sudan (p. 66) for fifteen to twenty minutes. After being washed, the sections may be mounted in levulose.

We will first study the paraffin sections. Under low power we notice transverse, oblique and longitudinal sections of seminiferous tubules.

Structure of Seminiferous Tubules.

They are lined by stratified epithelium, which differs in height and structure in the different tubules. The lumen is more or less completely filled by a thready, redstained mass, in which we distinguish numerous small, bright blue nuclei. This is the secretion of the testicle, i.e., its formed constituents, the **semen**.

In the interspaces between the tubules we recognize many blood-vessels, surrounded by masses of peculiar cells. These cells have been named **inter-mediate** or **interstitial cells** of the testicle.

Our picture shows under high power a small portion of our section. Two neighboring tubules are depicted in cross-section, showing a part of their wall and the interstitial substance between them. Each tubule is surrounded by a connective tissue **membrana propria**, which is mounted internally by **stratified epithelium**. The epithelium differs in the two tubules. In the left tubule we find, following the *membrana propria*, a simple layer of large cells, containing each a large nucleus. They are the **spermatogenia** (*spg*). Between them we see, at definite distances, cells with smaller nuclei, also mounted on the *membrana propria*, which, however,

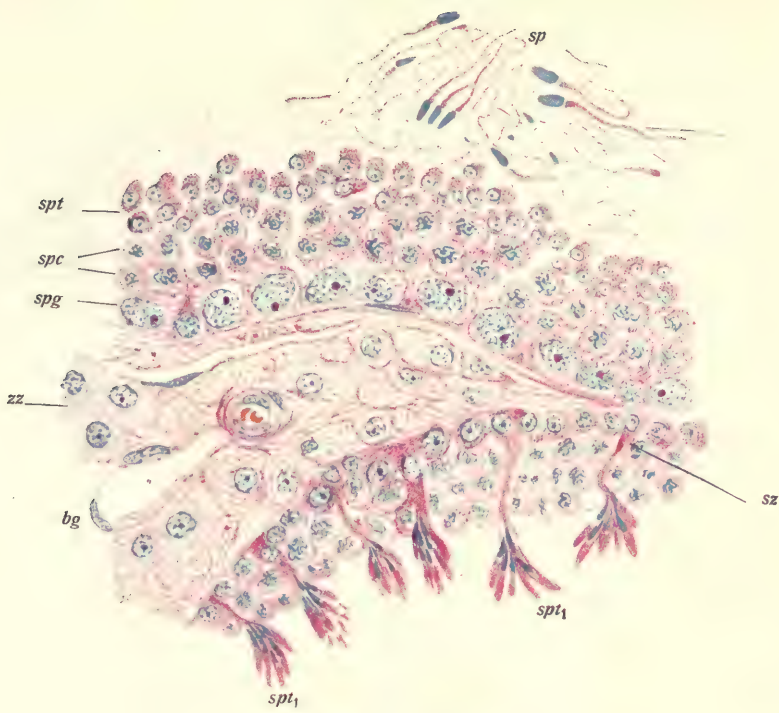


Fig. 147.



Fig. 148.



can hardly be distinguished from the spermatogenia during this state of spermiogenesis, taking place within their body. They are the so-called **foot-cells** or **cells** of **Sertoli**. The nucleus is smaller than that of the spermatogenia and slightly more irregular in shape, the cell-body is pyramidal or conical, the base resting upon the *membrana propria*, while the apex protrudes into the succeeding layer of cells.

Next to these we come to two or three layers of slightly smaller cells, the nuclei of which are in the state of division. They are the **spermatocytes** (*spc*). They are derived from the spermatogenia by indirect cell division, and dividing in turn (double division) furnish those cells which form the two to three innermost strata, the **spermatids** (*spt*). The latter are smaller in body as well as in nucleus than the spermatocytes and are more loosely connected. They furnish the actual **spermatozoa** by a complicated process of metamorphosis, affecting both nucleus and cell-body. The greater steps of this process can be traced in our specimen.

*Structure of the
Mature Spermatozoa.*

The lumen of our tubule contains a large amount of ripe **spermatozoa** (*sp*). They consist of a head, intermediate piece or neck and tail. The **head** appears at one time as an approximately oval plate, another time as a small wedge. In the former case we have a face-view, in the latter a side-view, so that the actual shape of the head is that of an oval plate, which tapers and becomes thinner in front. The **intermediate piece** or **neck** is slightly shorter, but much narrower than the head, and of cylindrical form. While the basophilia of the head proves its chromatin-nature, the neck as well as the tail take a red stain, viz., are acidophilic. Higher power still will reveal a disc-shaped formation on either end, remains of the central bodies of the spermatids. The axis is formed by the axis-thread, surrounded by a spiral covering, which continues through the *tail* of the spermatozoon, forming the main component of the latter. Neck and tail can hardly be separated, forming one continuous structure, as it were.

Spermiogenesis.

Looking at the section of the tubule at the right hand, different conditions present themselves. Again we recognize spermatogenia and spermatocytes, the spermatids (*spt*₁), however, have changed their shape and, what is still more striking, their position. The tendency of the cell to elongate, which we have previously noticed, has led to the development of a long-drawn-out formation, the anterior portion of which is occupied by the wedge-shaped nucleus. The chromatin has become so dense that it stains evenly blue. We have here young, immature spermatozoa. They are grouped in masses in such a manner that the cell-body points toward the lumen and the nucleus toward the *membrana propria* of the seminiferous tubule. Each of these bundles of young spermatozoa is approximated to the central end of a cell of *Sertoli*, which now cut a prominent figure, while previously they could hardly be recognized. A copulation, so-called, takes place between the young spermatozoa and the cells of *Sertoli*, which likely causes a better nutrition of the former.

Other fields will show us the further development of the immature spermatozoa and at the same time demonstrate the formation of new spermatids by the division of spermatocytes.

The interstitial substance is made up of large polyhedral cells, their bodies presenting a beautiful meshwork of protoplasm. Comparing our findings with a section stained in sudan, we find these interstitial cells filled with minute drops of a fatlike substance, taking a bright red stain. One specimen will supplement the other in a desirable manner.

Fig. 148.—Epididymis of Man

175. $\frac{3}{4}$. Formalin. Frozen section. *Biondi* solution.

The head of the epididymis is bisected with the razor, fixed in 10% formalin, and on the following day transferred to 5% formalin. Thin frozen sections are stained in *Biondi* solution (p. 67).

Our picture, taken from a section, made at right angles to the longitudinal axis of the testicle through the head of the epididymis, shows the two characteristic tubular sections of the epididymis, the ***ductuli efferentes*** (*def*) and the ***ductus epididymidis*** (*dep*).

Ductuli Efferentes.

In the efferent tubules the surface of the epithelium has become irregular, presenting numerous elevations and indentations. It is a simple, cuboid, ciliated epithelium. The cilia are not complete everywhere, forming short hanks, which project from the cells. The cells, especially those mounted on the projecting part of the mucous membrane, also contain in the portion next to the lumen larger or smaller masses of granules, which stain intensively orange, probably representing a pre-stage of the secretion. The outer covering of the tubule is formed by a connective tissue *membrana propria*. Between the tubules we also find abundant connective tissue.

Ductus Epididymidis.

The low cuboid is replaced by a high epithelium in the duct of the epididymis (*dep*), a cylindric epithelium, arranged in two rows and ciliated. The oval nuclei of the cylindric cells and the round nuclei of the so-called replacing cells together form a basal nuclear zone of the epithelium, in contradistinction to the non-nucleated central protoplasmic zone. The cilia are longer than those of the efferent tubules. The epithelium is mounted on a *membrana propria*, which gradually gathers some circular smooth muscle fibres.

PLATE 63

Fig. 149.—Ductus (vas) Deferens of Man

Fig. 150.—Human Prostate

Fig. 149.—Ductus (vas) Deferens of Man

25. $\frac{2}{3}$. Formalin. Frozen section. *Biondi* solution.

Pieces of the human vas deferens, 0.5–1 cm in length, are fixed in 10% formalin and transferred after twenty-four hours to 5% formalin. Frozen sections are stained in *Biondi* solution (p. 67).

Structure of the Ductus Deferens.

A main characteristic of the vas deferens is the extraordinarily well-developed **muscular coat**. Our specimen, taken from the first third of the duct, shows three distinct strata of smooth muscle fibres, a middle circular layer (*cm*) and an internal (*ilm*) and an external (*alm*) longitudinal layer. In the middle and last third of the duct the different strata are not sharply defined, but mingle. Externally the muscular layer is surrounded by a connective tissue **fibrous coat**, which is continuous with the adipose tissue of the spermatic cord. A part of the latter is seen in our specimen, containing **arteries** (*a*), **veins** (*v*) and **nerves** (*n*).

The mucous membrane is folded longitudinally in several places, projecting into the narrow lumen of the duct. It is lined with two layers of cylindrical epithelium (*ep*), which is lower than the epithelium of the duct of the epididymis and generally lacks the cilia. **Submucosa** (*sbm*) and **propria** (*pr*) are differentiated only with difficulty.

Fig. 150.—Human Prostate

150. $\frac{3}{4}$. Sublimate. Frozen section. *Biondi* solution.

Small pieces of the organ are fixed in 3% sublimate solution for four to five hours, washed overnight in running water and preserved in 5% formalin. Thin frozen sections are stained in *Biondi* solution (p. 67).

Structure of Prostate.

The prostate is an alveolar gland. Five of its roomy alveoli (*alv*) appear in section in our specimen. They are lined by a simple layer of epithelium, the cells of which are partly low cuboid, partly high cuboid, partly cylindrical. The small globular nucleus lies at the base. In superficial sections through the epithelium, which are not rare, owing to the extensive alveolar lumen, we notice that the cells are pentagonal in section. The cell-body is slightly swollen at the extremity near the lumen, containing a light net-formed protoplasm. The lower the cell, the deeper red it stains, appearing



Fig. 149.



Fig. 150.

filled with numerous fine granules. These granules are also found in the secretion (*sc*); they are seen in large masses in the lumen of the alveoli.

A great amount of smooth muscle is characteristic of the prostate, being found among the connective tissue, separating the alveoli; our specimen shows them united in bundles, partly longitudinal (*ml*), partly transverse (*mg*). The connective tissue between the bundles is very scanty. Around the alveoli it becomes denser, forming a thin *membrana propria*.

PLATE 64

Fig. 151.—Human Penis

Fig. 152.—Ovary of the Cat with Tube and Fimbria

Fig. 151.—Human Penis

12. $\frac{3}{4}$. Formalin. Frozen section. Resorcin fuchsin. Paracarmin.
Picroindigcarmin.

The organ is divided into pieces of 5–10 mm thickness, which are first subjected to 10% formalin for twenty-four hours and thereafter placed in 5% formalin for an equal length of time. Frozen sections are made from the different regions and stained primarily in resorcin fuchsin (p. 63) for fifteen minutes. Wash thoroughly in 95% alcohol, transfer to paracarmin (p. 55), wash in 70% alcohol and counterstain in picroindigcarmin (p. 67). After ten minutes wash again in 70% alcohol, dehydrate in absolute alcohol and after the use of xylol mount in Canada balsam. We will confine ourselves to the description of a section through the cavernous portion.

The Component Parts of the Penis.

Our picture represents the lower portion of such a section. Of the three component corpora the **corpus cavernosum urethrae** (*ccu*) is in full view, while the **corpora cavernosa penis** (*ccp*) are only partially visible. The latter two are surrounded by a powerful connective tissue **albuginea** (*alb₁*). It contains numerous elastic fibres, forming a network, which have been stained black by the resorcin fuchsin. The two lateral albuginae unite in the centre to form the **septum penis** (*sp*). The latter is interrupted by numerous gaps (*anast*), through which the two corpora communicate.

The **corpus cavernosum urethrae** (spongiosum) follows below. It is likewise surrounded by an albuginea (*alb₂*), which, however, is much thinner than that of the penile corpora cavernosa and can be differentiated from the latter without difficulty. Larger and smaller blood-vessels (*bg*) and nerves (*n*) are found between the corpora. The arteries especially with their strong muscular coat are very striking, carrying large amounts of elastic fibres in their adventitia.

The structure of the three parts of the penis is in general the same. Connective tissue septa arise from the albuginea, which form an interlacing framework, resulting in a widely distributed, spongy, cavernous system, into which the afferent blood-vessels empty. The vascular spaces in the corpora cavernosa penis are considerably larger than those of the corpus cavernosum urethrae (spongiosum); the septa are also thicker in the former.

While the albuginae of our specimen, aside from nuclei and elastic fibres, stain almost purely blue, the septa of the corpora take a green color. High power proves this to be due to deposits of **smooth muscle fibres**. They



Fig. 151.



Fig. 152.

again are better developed in the corpora cavernosa than in the corpus spongiosum.

Within the septa we see a large amount of blood-vessels (*bg*). The small arteries not infrequently show a thickened intima.

The vascular spaces are lined by a simple layer of **flat epithelial cells**.

Male Urethra.

Within the corpus cavernosum urethræ we see the cross-section of the **urethra**. The mucous membrane is arranged in numerous longitudinal folds, giving the lumen the appearance of a narrow transverse cleft with numerous sinuses. The **epithelial lining** (*ep*) is composed of stratified cylindric epithelium, thus differing from that of the urinary bladder and the ureter. It is mounted on a connective tissue **propria** (*pr*), which merges into the **submucosa** (*sbmc*) without any definite border-line. The submucosa in turn becomes continuous with the tissues of the corpora.

In the submucosa of the urethra we find numerous glands, known as urethral glands or **Littre's glands** (*ldr*). In our section they extend far into the substance of the corpus spongiosum, other sections will show them superficially in the propria. They open into the sinuses of the urethra and are lined by cylindrical epithelium, the cells of which contain the granular pre-stage of the secretion.

Fig. 152.—Ovary of the Cat with Tube and Fimbria

18. $\frac{3}{4}$. Sublimate—*Mueller's* solution—acetic acid. Paraffin section.
Biondi solution.

The ovary of the cat, which we have selected for our study, is very similar in structure to the human ovary, offering a better survey than the latter, due to its smaller size. The ovary of one side is removed with its tube and fixed on a wax plate with porcupine bristles in the natural position. The specimen is fixed for six hours in the mixture of sublimate, *Mueller's* fluid and acetic acid, discussed on page 31. The other ovary is taken out separately, cut in half and fixed in the same manner. After completed fixation wash in running water overnight and dehydrate by the usual grades of alcohol. After the specimen has hardened in 95% alcohol, it is removed from the wax plate, dehydrated thoroughly and embedded in paraffin. The sections are made to include ovary, tube and fimbria at the same time. They are stained in *Biondi* solution (p. 67).

Our specimen shows the large, egg-shaped, longitudinal section of the ovary, continued at the left in a thick stem, the ovarian ligament; it connects the ovary with the uterus. Above the former appears the **Fallopian tube** (*tu*) in cross-section. The ovary, or the aforementioned ligament respectively, is connected to the tube by a fold of peritoneum, the **mesovarium**. The tube runs about parallel to the anterior border of the ovary, ending laterally in the **fimbria** (*fi*). The **osteum abdominale** (*oa*) of the latter appears at the right. Owing to its curved course, the Fallopian

tube as well as the mesovarium appear in transverse section, the latter connecting the tube and the fimbria.

Ovary.

The ovary presents two zones, the **medullary** (*mas*) and the **cortical** (*rs*) substance. The former enters the organ at the *hilum* in the form of powerful connective tissue, containing numerous vessels, starting at the point of junction of the ligamentum ovarii proprium and the ligamentum latum. It forms the foundation of the organ. It is surrounded by the cortex, which even to the unaided eye appears characterized by larger and smaller empty spaces, lined by epithelium, the **Graafian follicles**. The follicles vary in size, the smaller and medium sizes (*pfo* and *fo₁*) are solid, the larger (*fo₂* and *fo₃*) enclose a more or less spacious hollow, the **follicular hollow**. Each follicle contains an ovum.

Fallopian Tube.

The tube (*tu*) presents distinctly a strong **circular layer of muscle**, bounded internally by the *propria*. The latter forms many folds, projecting into the lumen and being surmounted by secondary projections. They are partially cut off in our specimen and appear free in the lumen. The folds are covered by **epithelium**.

Fimbria.

The fimbria (*fi*), the funnel-shaped, abdominal extremity of the tube of course presents essentially the same structure as the wall of the tube. Its mucous membrane lies free in the abdominal cavity.

PLATE 65

Fig. 153.—Ovary of the Cat

Fig. 154.—Fallopian Tube of the Child

Fig. 155.—Fallopian Tube of the Child

Fig. 153.—Ovary of the Cat

80. Sublimate, *Mueller's* fluid-acetic acid. Paraffin section. *Biondi's* solution.

Primary Follicle.

A field of the preceding specimen is examined under high power. The follicles appear in almost all stages of development. Near the surface we find numerous primary follicles (*pfo*). They contain one ovum each, a large cell with a globular nucleus and light red, meshy protoplasm. The nucleus shows a distinct chromatin frame and a small nucleolus. The ovum-cell, about 50–60 μ in diameter, is surrounded by a simple layer of low, cuboid cells, the **follicular cells** (*fo*₁). The follicles are separated by the connective tissue of the ovarian cortical substance, which is extremely rich in fibroblasts.

Growth of the Follicles.

Farther inward we find more mature follicles (*fo*₂) of about twice the size of the preceding. The enlargement seems mainly due to a proliferation of the follicular epithelium, which has become stratified. The ovum presents no essential changes, but becomes more distinctly isolated from the follicular epithelium, having acquired a red-stained coat, the **zona pellucida**. The entire follicle, too, is better differentiated from its surroundings, taking on a connective tissue capsule, the **theca folliculi**.

The further enlargement of the follicle (*fo*₃) is not due to an increase in number, but in size of the cells of the follicular epithelium; they become cuboid and cylindrical. The ovum, too, has attained a considerable size, measuring now over 100 μ in diameter. The protoplasm of the egg presents an exquisite net structure, the zona pellucida has become much more distinct.

The last stage of follicular development (*fo*₄), shown in our specimen, presents an almost ripe follicle. The **intrafollicular space** has become filled with a fluid, the **liquor folliculi**, a part of the newly formed follicular cells having undergone liquefaction. This process can be observed on favorable places in our specimen. Externally, surrounding the entire follicle, we have the **theca folliculi**. It is composed of vascular connective tissue, blended with numerous strands and nests of peculiar cells, the **interstitial cells** or **grain cells** (*iz*). Just as we found the testicle of the cat studded with an abundant amount of interstitial cells, so do we observe the same in the ovary. They are polyhedral cells, differentiated distinctly from the red connective tissue by their orange-yellow color, taken from the *Biondi's* solution. The different nests of cells are separated by connective tissue fibres. The latter form an essential constituent of the theca folliculi, so that it can only with difficulty be distinguished from the surrounding tissue. In the mature human ovary they are less well developed; consequently the fol-

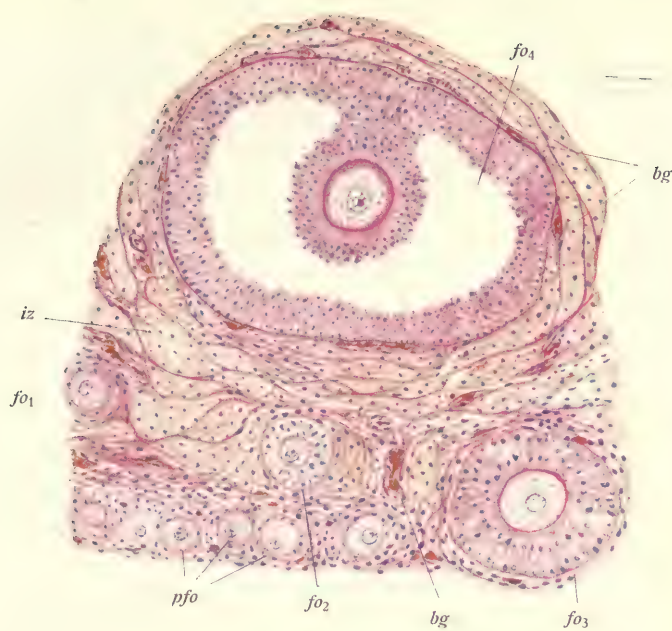


Fig. 153.



Fig. 154.

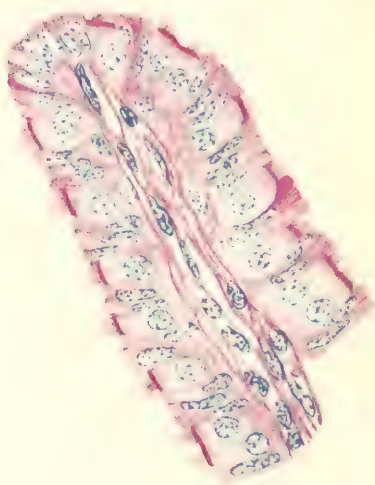


Fig. 155.

licle can easily be differentiated from the adjacent tissues. The cells are wanting in the outer portion of the cortex. Internally the theca folliculi is bounded by a structureless, bright red membrane, the **choroid membrane**. The blood capillaries (*bg*), coming from without, pierce as far as this membrane. The *follicular epithelium* lines the follicle in several layers and at one place projects in form of an elevation into the follicular cavity. This **cumulus oophorus** contains the ovum. The follicular cells have partly cuboid, partly cylindrical form. The latter predominate especially around the ovum. The ovum itself is covered by the now fairly thick *zona pellucida*, which under very high power shows a fine radiating striation. The diameter has grown, measuring now about 150 μ . The nucleus of the ovum, the **germinal vesicle** so-called, shows a distinct chromatin net and a well-developed chromatic membrane. It possesses a strikingly large nucleolus, which has been called the **germinal spot**. The formerly very distinct net-structure of the protoplasm of the egg has become slightly blurred, due to a deposit of deutoplasmatic substance, the **yolk granules**. Toward the zona pellucida the protoplasm becomes more homogeneous, forming the **yolk cortex**.

During the further development of the ovum the follicle becomes larger, pushing the younger follicles aside, and finally gains the surface of the organ. The ovum, leaving the bursted follicle, does not attain its complete maturity and capability of fertilization until its entrance into the tube.

Fig. 154.—Fallopian Tube of the Child

60. $\frac{3}{4}$. Formalin. Frozen section. *Biondi* solution.

Transverse sections of the organ are fixed in 10% formalin and transferred on the following day to 5% formalin. Thin frozen sections are stained in *Biondi* solution (p. 67).

Structure of the Oviduct.

A pronounced formation of folds is characteristic of the mucous membrane of the Fallopian tubes. They are longitudinal folds, which in the ampulla, as shown in our specimen, are very high and surmounted by secondary folds, so that the entire lumen is finally filled with a contorted mass of folds. In the uterine portion of the tubes the folds are very poorly developed. The folds are covered by **epithelium**, to be described shortly, and contain as fundamental substance a thin vascular, connective tissue *propria*, which at the base of the folds is mounted in a thin layer upon the muscularis. A submucosa cannot be distinguished. The **muscularis** of the infantile tube is weakly developed, but still illustrates a main bulk of circular smooth muscle fibres. Externally to the circular layer we find, although very poorly developed and often interrupted, a stratum of longitudinal muscle fibres, appearing most pronounced near the junction of the mesosalpinx. Following the muscular coat we come to a loose connective tissue **subserosa**, and finally to the **serosa** proper.

Fig. 155.—Fallopian Tube of the Child

550. $\frac{3}{4}$. Formalin. Frozen section. *Biondi* solution.

Tubal Epithelium.

To study the tubal epithelium, we adjust the summit of one of the folds under high power. We recognize a peculiar ciliated epithelium—peculiar inasmuch as ciliated cells alternate with non-ciliated ones. The epithelium is simple cylindrical. The ciliated cells are conspicuous by their size, their light cell-body and, finally, by an intensely red, basal membrane. The nucleus, most often found in the centre, but occasionally nearer the base of the cell, is almost completely globular. The deep red basal border is in striking contrast to the light cell-body, a large number of cilia projecting from the former. If we use still greater power, we are able to discern the composition of the basal border of closely approximated basal corpuscles. Only seldom do we find two ciliated cells together; they are generally separated by a non-ciliated cell. The latter are much narrower than the ciliated cells; the nucleus varies in shape, but most often is elongated and generally has a superficial position. Their club-shaped heads project very often between the ciliated cells. They are usually darker in color and often present secretory granules in their club-shaped extremity. Very probably they are secreting cells, which have formerly been ciliated cells.

PLATE 66

**Fig. 156.—Transverse Section through the Body of Uterus of
the Child**

Fig. 157.—Uterine Mucous Membrane of the Child

Fig. 156.—Transverse Section through the Body of Uterus of the Child

10. $\frac{2}{3}$. Formalin. Frozen section. Cresyl violet.

Excellent specimens are furnished by the uterus of children during the first weeks of life. The organ is separated from its appendages and removed, including a piece of the vagina. With the razor it is divided into three transverse sections, an upper, middle and lower part. After fixing in 10% formalin for twenty-four hours, the specimen is placed in a 5% solution, where it remains for two days. Frozen sections can now be made. A disk, 2–3 mm in thickness, is cut from the upper third and transverse sections made thereof, which are stained in cresyl violet (p. 62).

Three layers can immediately be distinguished in the wall. Next to the lumen we find the **endometrium**, following this the muscular **myometrium** and, enveloping the latter, the thin **perimetrium**.

Structure of the Body of the Uterus.

The uterine lumen is a narrow transverse cleft, running in our picture from the upper left to the lower right. Numerous lateral sinuses branch off, which are covered with fingerlike hollow sprouts, the **uterine glands** (glandulæ uterinæ, *dr*). They are fairly short in the infantile uterus, growing much larger in later years, so that they reach or even project into the myometrium. The lumen of the uterus is lined by the **uterine epithelium**, dipping into the uterine glands. The epithelium is mounted on a powerful connective tissue **propria** (*pr*), which, especially right below the epithelium, is very rich in cells, creating in our specimen a dark, reddish violet zone. The *propria* contains numerous smaller vessels. It rests directly on the myometrium without any interposed submucosa.

The myometrium of the infantile womb is ill-developed, increasing in thickness as the organ grows larger, occupying the greater part of the wall at the time of puberty. Underneath the *propria* we find here only scanty bundles of smooth muscle fibres, which have been cut transversely and therefore represent longitudinal fibres in the uterus; they make up the **stratum submucosum** (m_1). The bulk of the muscular layer is represented by the subsequent **stratum vasculare** (m_2). It consists of interlacing circular muscle bundles, between which some longitudinal bundles are found. As its name indicates, it is characterized by an abundant supply of large blood-vessels. More externally we come to the **stratum supravasculare** (m_3), which is much narrower than the former and contains besides the circular quite a number of longitudinal bundles. Among the connective tissue

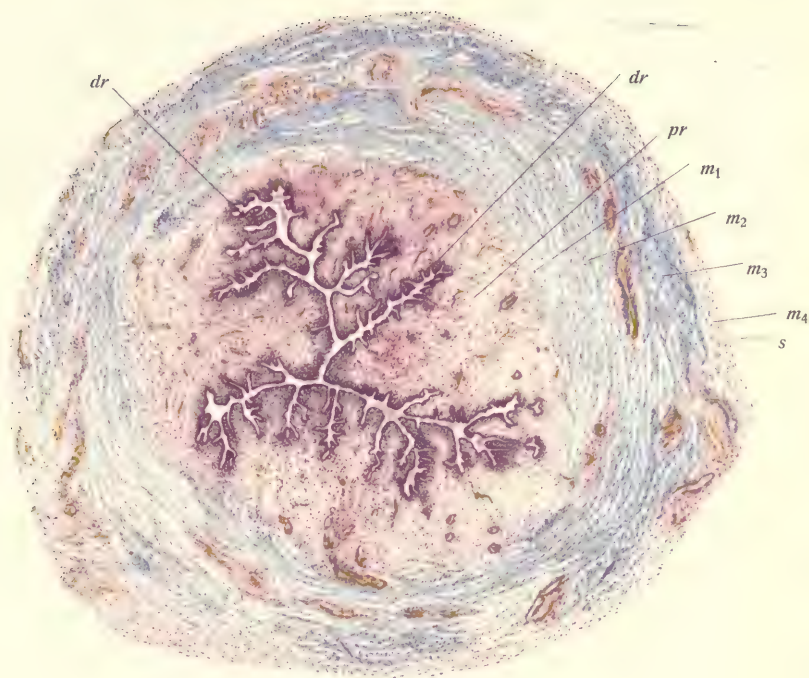


Fig. 156.

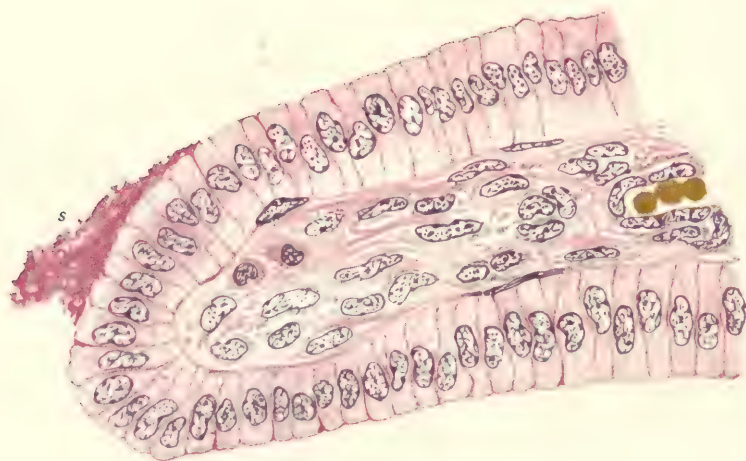


Fig. 157.

of the periphery we recognize some very irregularly distributed, longitudinal, muscular fibres, which make up the last layer, the **stratum subserosum** (m_4). The entire muscular coat of the uterus is composed of smooth muscle cells, which are short in the virgin uterus, but attain a considerable size during pregnancy.

Externally the wall of the uterus is bounded by the simple flat epithelium of the serous coat (s), which is joined to the myometrium by scanty subserous tissue.

Elastic tissue does not appear in the infantile uterus, stained with resorcin fuchsin, except in the vascular walls. This is not so later on. The mature uterus contains a considerable amount of elastic fibres in the myometrium. They surround the muscular bundles and form an elastic membrane against the serosa.

Fig. 157.—Uterine Mucous Membrane of the Child

600. Formalin. Frozen section. Cresyl violet.

Structure of the Uterine Mucous Membrane.

We will now examine the uterine mucous membrane under high power. The epithelium is simple cylindric, composed of moderately broad and high cells. Our stain has colored the protoplasm reddish violet, the intensity of the red increasing as we near the glands, although the shape of the cells remains the same. Evidently the cells are mucous in character, with a view to our fixing agent, although no pre-stage in form of granules can be demonstrated in the cells. The secretion proper (s) is seen in the form of homogeneous, red masses throughout the lumen.

At the age of puberty the epithelia undergo a change, inasmuch as they acquire cilia, which also appear in the uterine glands. This condition is not constant.

The epithelium rests on a distinct **basal membrane**, containing always some connective tissue cells.

In the *propria* we find irregularly arranged connective tissue fibres. Between the latter we notice numerous connective tissue cells and migrating cells.

PLATE 67

**Fig. 158.—Longitudinal Section through the Cervix Uteri of the
Child**

Fig. 158.—Longitudinal Section through the Cervix Uteri of the Child

10. $\frac{3}{4}$. Formalin. Frozen section. Cresyl violet.

The lower third of the uterus is used for the preparation of longitudinal sections. A longitudinal median incision divides it into a right and left half. One half is placed on the freezing table, cut surface uppermost, and sections of moderate size are made, which, of course, divide into an anterior and a posterior half. Staining of the sections in cresyl violet (p. 62).

Structure of the Cervix Uteri.

Fig. 158 demonstrates the posterior half of such a section. At the left we have the **cervical canal** (*ck*), to the right *Douglas's space* (excavatio recto-uterina). Below we have the **portio vaginalis** of the cervix extending into the vagina (*vg*). Our specimen shows naturally only the small posterior lip (labium posterius). Behind it the **fornix** (*fo*) extends quite a distance upward.

The arrangement of layers is the same as in the body of the uterus. The **endometrium**, which had been smooth in the body, forms transverse folds in the cervix, the **plicae palmatae**, some of which can be seen in the upper part of our specimen (*plp*). They are more or less high and have numerous secondary elevations. Toward the external os the folds disappear gradually and instead we find deep sinuses in the uterine mucous membrane, which extend high up in the mucous membrane (*cdr₁*), the **cervical glands** (*cdr*). They are found throughout the cervix, but attain their greatest development at this point. The *epithelium* is simple cylindric, showing no cilia in this case. It is continuous with the epithelium of the cervical glands, the secretion of which, a tenacious mucus, can be seen at different places (*cdr₁*) in the lumen of the gland, appearing as a red mass.

The **propria** (*pr*) offers the same conditions as that of the uterine body.

The different muscular layers of the **myometrium** (*mm*) can only be differentiated with difficulty (*m₁-m₄*); the thin longitudinal fibres of the stratum subserosum (*m₄*), however, is very nicely demonstrated. The vaginal portion consists mainly of propria tissue mingled with longitudinal muscular fibres. The circular muscles form a powerful layer at the posterior wall toward the fornix. The longitudinal fibres of the stratum supravasculare (*m₃*) and the stratum subserosum are continued in the vaginal wall, where they form the main bulk of the **muscularis vaginae**. Near the lumen of the vagina we recognize a layer of circular fibres, which may be regarded as a continuation of the stratum vasculare.

The **serosa** (*s*) is closely mounted on the myometrium, consisting of a



Fig. 158.

simple layer of epithelium and of connective tissue, the latter especially well developed in the lower parts.

*Structure of the
Vaginal Mucous Membrane.*

The foundation of the vaginal mucous membrane is formed by a *propria*, which shows similar conditions to that of the uterus. It forms high circular folds, the ***columnae rugarum*** (*cr*), which again has secondary folds. The ***epithelium***, filling out the depressions between the folds, is a powerful, stratified, flat epithelium. In the fornix it is reflected over the portio vaginalis, investing its outer surface, and at the external os merges into the simple cylindrical epithelium of the uterine mucous membrane (X).



PLATE 68

**Fig. 159.—Transverse Section through the Superior Rectus
Oculi of the Sheep**

**Fig. 160.—Longitudinal Section through the Superior Rectus
Oculi of the Sheep**

6. THE ORGANS OF MOTION

Fig. 159.—Transverse Section through the Superior Rectus Oculi of the Sheep

35. $\frac{3}{4}$. Formalin. Frozen section. Crèsyl violet.

To demonstrate the composition of an entire muscle, we may use cross as well as longitudinal sections. The muscle should, of course, be as small as possible, in order to give a better survey; furthermore, the fibres should be as parallel as possible, in order to obtain purely cross and longitudinal sections respectively. The straight muscles of the eye are especially adapted for this purpose. For reasons which we will appreciate later, we select the muscles of the sheep. After removing the skullcap and the brain from a fresh sheep's head, we take off the entire roof of the orbit by sawing through at both the lateral and the median boundaries. The two lines of section converge posteriorly. With a chisel the entire roof may now be broken off, revealing the orbital contents. The muscles are quickly dissected from the orbital fat, detached at their origin and insertion, spread with slight tension on a suitable wax plate and fixed in 10% formalin. The following day they are placed in 5% formalin and then are divided into transverse and longitudinal sections on the freezing microtome. The sections are stained in cresyl violet (p. 62) and mounted in levulose.

Structure of the Muscle.

Such a transverse section shows the muscle surrounded on all sides by a more or less powerful connective tissue stratum, the **perimysium externum** (*pe*). The latter sends connective tissue trabeculæ into the interior of the muscle, which divides its mass of contractile fibres into larger or smaller bundles. The septa of connective tissue, separating the muscular bundles, are collectively termed the **perimysium internum** (*pi*). Connective tissue strands leave the latter, piercing into the muscle bundles, where they separate the different muscular fibres. In the muscles of the skeleton this arrangement is more orderly, inasmuch as there the smallest primary muscular bundles unite to form larger ones, which are separated by coarser connective tissue septa; these secondary bundles form very large, tertiary bundles. This typical arrangement is not present in the muscles of the eye. Here we find, especially at the periphery of our specimen, that the muscular fibres do not form bundles, but are scattered about in the connective tissue.

The blood-vessels (*a* and *v*) and the nerves (*n*), supplying the muscular bundles, are found in the perimysium internum. The nerves distinguish them-

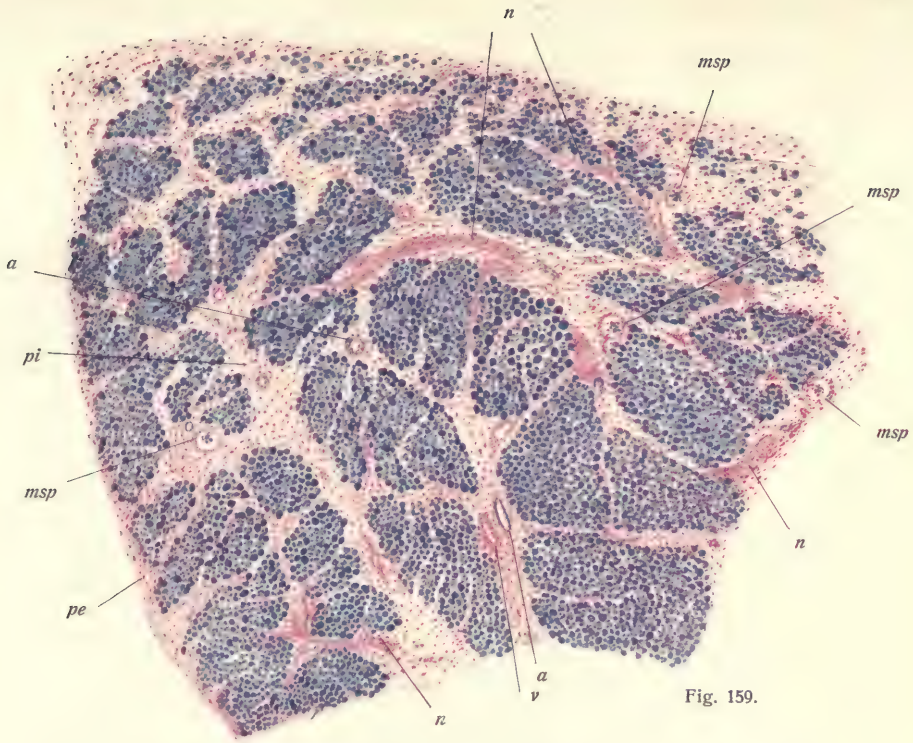


Fig. 159.

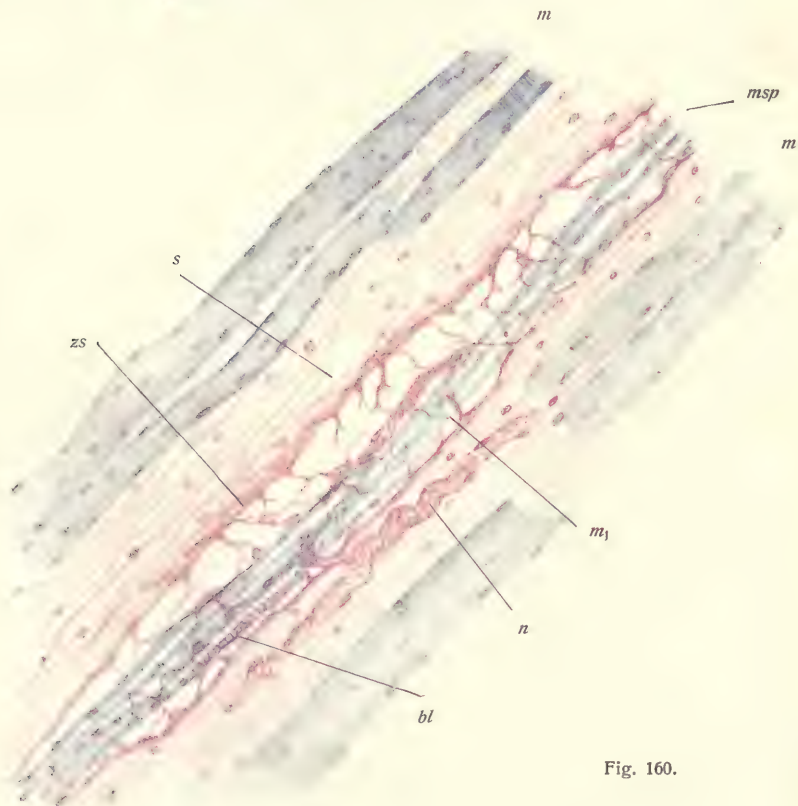


Fig. 160.

selves by their deep red color from the light red connective tissue; in thick sections they can be traced for long distances.

There are other structures of interest found in the perimysium internum. We find small, completely round, hollow spaces, surrounded by a distinct capsule, enclosing in their interior several transverse sections of muscle and nerve-fibres, which, however, do not occupy the hollow space completely. Very often the spaces are absolutely empty. In those cases the contents have fallen out. Very interesting formations present themselves in the so-called **muscle spindles** (*msp*). They are present in small amounts in all skeleton muscles, but they are wanting in the ocular muscles of man and many animals. In the sheep they are found in large amounts in the muscles of the eye. The structure of these interesting features are better illustrated in the longitudinal than the transverse section.

Fig. 160.—Longitudinal Section through the Superior Rectus Oculi of the Sheep

145. $\frac{3}{4}$. Frozen section. Cresyl violet.

Muscle Spindles.

Thin longitudinal sections through the muscle immediately present the spindles. We can trace them for a long distance, since they attain a length of over 10 mm. Each spindle during its course shows one or more enlargements and is surrounded by the **spindle-sheath**, which is lamellar in structure, as we can see in the cross-section better than in the longitudinal section. Fibroblasts are scattered between the connective tissue lamellæ. Externally the sheath is continuous with the perimysium internum. Internally a continuous layer of branching fibroblasts forms a layer, their processes anastomosing within the interior of the spindle. The muscular fibres (*m*₁) run along the axis of the spindle, entering and leaving the spindle in different numbers. They do not always leave and enter at the extremities, but, especially in spindles, having several enlargements, they may enter and leave at the sides. The fibres show a very characteristic arrangement within the spindle. They branch and anastomose, forming a **muscular network** within the spindle. The fibres composing the net are very narrow; at one place we find closely packed, globular or slightly compressed vesicles (*bl*) deposited, which seem to be altered muscle nuclei. More or less, at least two, medullated nerve-fibres (*n*) enter each spindle, sensory and motor in character, forming their characteristic endings. In all probability the interior of the spindle is filled with an albuminous fluid. The physiological significance of these spindles is still in question; it is probable, however, that they are important perceptory end-organs.

PLATE 69

Fig. 161.—Nerve-Ending in the Muscles of the Common European Viper (*Tropidonotus Natrix*)

Fig. 161.—Nerve-Ending in the Muscles of the Common European Viper (*Tropidonotus Natrix*)

62. $\frac{3}{4}$. Gold chloride. Formic acid.

In order to get an idea of the manner and form of the nerve-endings in the striated muscles, it becomes necessary to select short as well as very thin flat and therefore transparent muscles. Of the small mammalia we may use the ocular muscles, the diaphragm and the intercostal muscles, but still better results are obtained with certain muscles of snakes, which are prepared in the following manner: A decapitated, large viper is cut with the bone forceps into several pieces, each about 10 cm in length. One such segment is placed upon its belly-surface in a wax- or paraffin-lined glass dish (photographic trays, 9×12 , are very suitable), and the skin of the back is split in the median line. After dividing the fascia, the skin can be stripped down on either side and is spread and fastened on the wax floor. We will now notice on both sides a row of closely approximated small muscles, 20–30 mm in length, which arise from the transverse processes, running obliquely toward the abdominal integument. Due to our method of preparation, they are very much stretched, and we are able to distinctly recognize thin nerve-trunks, which enter and cross through the muscles. At their origin the muscles are approximately cylindrical, but they become broader and thinner as they approach their insertion, finally entering the skin in parallel fibres.

The vital methylene as well as the gold method of staining are excellent for the demonstration of the nerves. We select the gilding process. Primarily the specimen is submerged in 100–200 cm³ of a 25–30% solution of formic acid, which is allowed to act for fifteen to twenty minutes. During that time we seek to remove the connective tissue, separating the muscles, and the air-bubbles contained therein, by the aid of a blunt glass needle. The formic acid is now decanted and substituted by the gold solution—200 cm³ of a 0.25–0.50% solution of gold chloride will suffice. By moving the vessel we make sure that every part of the specimen is in contact with the solution and, after covering the vessel, we leave the specimen for one hour. The excess solution of gold is removed by thoroughly rinsing with water and 200 cm³ of 25% formic acid are substituted, wherein the specimen remains overnight in the dark. The next morning the specimen will appear dark brownish violet; it is now placed in a mixture of 2 parts of 25% formic acid and 1 part of glycerin. The following day we use equal parts of glycerin and water, the next day one part of water to two parts of glycerin, and finally pure glycerin. To prepare a specimen, we separate with the aid of scissors one muscle in its entire length. The superfluous glycerin is al-

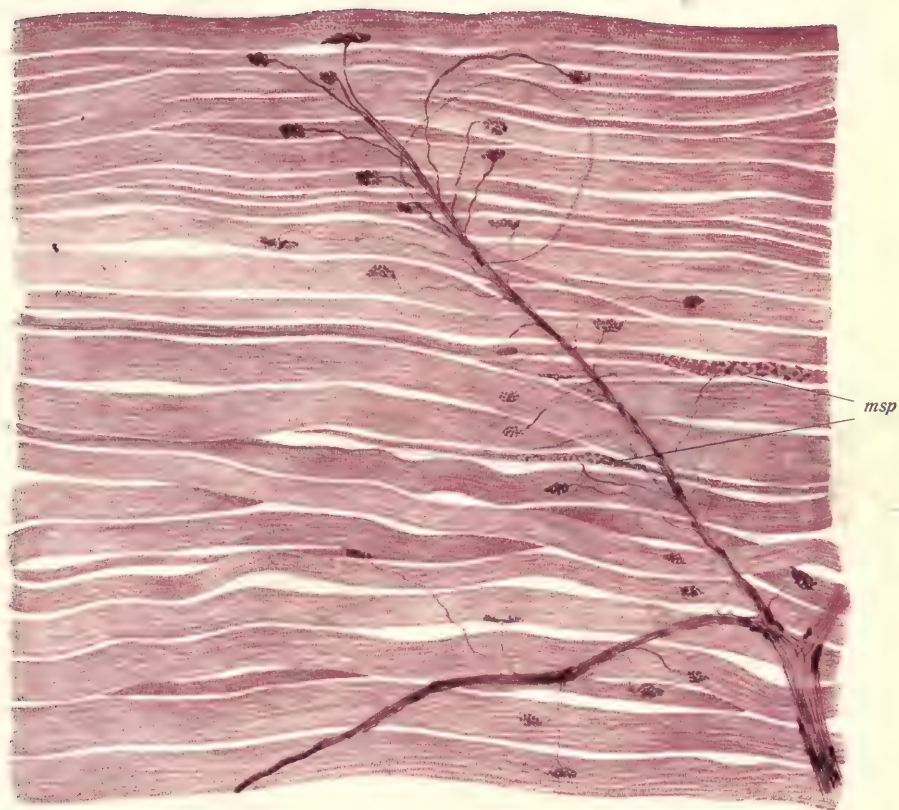


Fig. 161.



lowed to drop off and the muscle spread evenly on a cover-glass and mounted in levulose. In order to obtain a nicely spread and thin specimen, we place a lead ball on the cover-glass, which is left until the following day, when the cover-glass is surrounded with a border of cover-glass cement (p. 77).

*Distribution and Ending of
the Nerves within the Muscle.*

Our specimen gives a plain view of the course and the ending of the nerves in the muscle, and besides furnishes a good picture of the muscular structure. Our picture shows the ending of a nerve-trunk, after entering a muscle. It gives off lateral branches and finally breaks up in terminal branches. Each nerve-fibre, after a shorter or longer course, enters a muscular fibre, where it ends in a **motor end-plate**. The latter are seen partly in surface, partly in profile view. We are able to ascertain that each muscle fibre is provided with only one motor end-plate.

Aside from the motor endings, our specimen also presents sensory endings, which have been termed **muscle spindles** (*m_{sp}*), although their structure has nothing in common with the homonymous formations of the mammalia. These endings are only found in very thin muscular fibres. The nerve-fibre breaks up into delicate threads, which surround the muscle fibre for a long distance, being studded with platelike enlargements.

PLATE 70

Fig. 162.—Motor End-Plates from a Muscle of the Viper

Fig. 162.—Motor End-Plates from a Muscle of the Viper

600. $\frac{3}{4}$. Gold chloride. Formic acid.

Structure of the Motor End-Plates.

A favorable field is selected from the preceding specimen and studied under high power. Three muscle fibres, each supplied with a motor end-plate, appear in the field. Of the latter we find the middle one in face view, the lower in complete and the upper one in partial lateral view. A separate nerve-fibre leads to each end-plate. Their axis-cylinder appears almost black, but shows a very indistinct fibrillation. The medullary sheath remains unstained, but the sheath of *Schwann*, with its numerous nuclei, has taken a distinct light brown color and appears swollen in some places. The nerve-fibres are frequently accompanied by pigment cells, one of which can be seen at the upper extremity.

When nearing its end-plate, the nerve-fibre undergoes striking changes. The axis-cylinder becomes considerably thinner and frequently, not always, divides into two terminal branches. The medullary sheath disappears, and the sheath of *Schwann* becomes closely approximated to the axis-cylinder. On entering the end-plate the sheath of *Schwann* is lost, i.e., it becomes continuous with the substance of the end-plate. The latter consists of two parts, corresponding to the terminal branches of the axis-cylinder, the so-called *deer's antlers*, and a nucleated, protoplasmatic mass, containing the former, the **granulosa** or **sole-plate**. The end branches of the axis-cylinder show the most different forms and types, very often presenting a frappant similarity to a stag's antlers. The main dimensions are in a plane, however, the side view proves, that some nodular processes leave the different horns and pierce into the depth of the sole-plate. The granulosa is simply a mass of nuclear sarcoplasm, surrounding the antlers and the numerous processes thereof. Below it borders on the muscular columns, becoming continuous with the sarcoplasm-septa, dividing the columns; it never extends beyond the terminal processes of the antlers. The nuclei are more rounded than the nuclei of the sarcoplasm proper. Externally the sole-plate is bounded by the sarcolemma, respectively the sheaths of *Schwann* of the nerve-fibres, which blend with the sarcolemma.



Fig. 162.

PLATE 71

Fig. 163.—Sensory Nerve Endings on the Fibres of the Superior Rectus Oculi of the Sheep

Fig. 164.—Nerve Endings in the Tendon of the Superior Rectus Oculi of the Sheep

Fig. 165.—Transverse Section through a Small Branch of the Oculomotor Nerve of the Sheep

Fig. 163.—Sensory Nerve Endings on the Fibres of the Superior Rectus Oculi of the Sheep

145. $\frac{3}{4}$. Vital methylene blue staining.

As mentioned above, the vital methylene blue staining process furnishes very good demonstrations of the nerve endings in muscle, being the method of choice for the staining of sensory nerve endings, in preference to gilding. Any larger animal—rabbit, cat, etc.—may be used for this purpose, the injection taking place in the manner described on p. 60. About half an hour after the injection the cranium is opened and, after removing the roof of the orbit, the different ocular muscles are excised and spread on wax plates. A correspondingly large window is cut from the wax plate from below, and the specimen placed in a moist chamber. Every ten to fifteen minutes we control the progress of the staining, which should have attained its maximum intensity after one to two hours. After that we fix in a 10% solution of ammonium molybdate (p. 62) overnight, wash in running water for two hours, and, after dehydrating in cold alcohol for several hours, transfer to xylol. While in the 95% alcohol the specimen is taken from the wax plate. The muscles, especially in their distal parts, become so transparent that the entire as well as smaller pieces of the muscle can be mounted in Canada balsam.

*Ending of the Sensory
Nerves in Muscle.*

Aside from innumerable motor end-plates, located in the proximal thicker parts of the muscle, we find the sensory nerves winding around the muscular fibres for long distances in spiral fashion. Not infrequently they divide, and now and then give off small side branches, which, studded with buttonlike enlargements, end in the muscle fibre after a short course.

Fig. 164.—Nerve Endings in the Tendon of the Superior Rectus Oculi of the Sheep

145. $\frac{3}{4}$. Vital methylene blue staining method.

*Relation Between Muscle
and Tendon Fibres.*

The previous specimens also give us an opportunity to become acquainted with the relation between muscle (*m*) and tendon (*s*) fibres. The muscle fibres stain weakly with the vital process, but still sufficiently to bring out their transverse striation very distinctly. We notice how each muscle fibre ends in

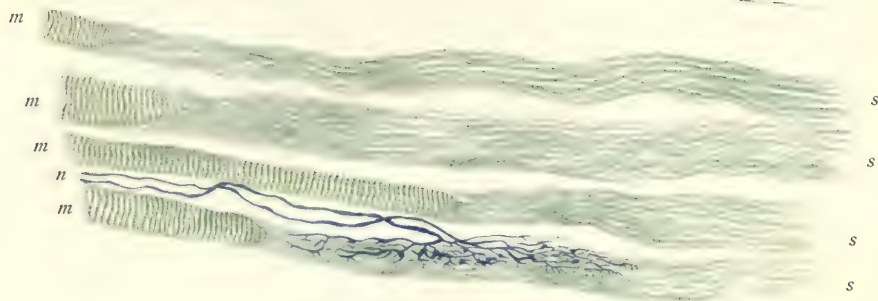


Fig. 164.

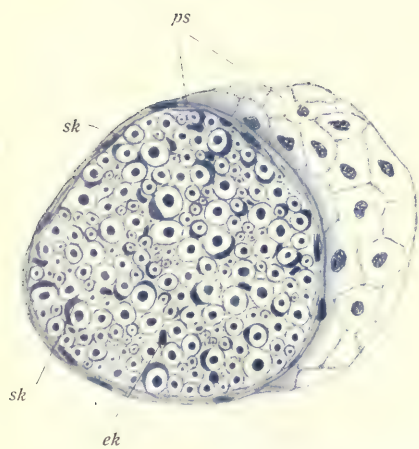


Fig. 165.

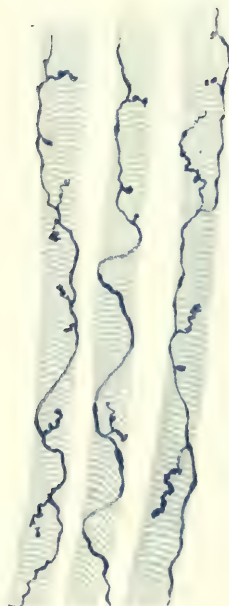


Fig. 163.

a conical point and how the tendon fibrils surround this conical end on all sides, being tightly cemented to the sarcolemma.

*Ending of the Sensory Nerves
in the Tendon.*

In the tendons we find nerve-endings, which are, of course, sensory. Our specimen shows one of these nerve-endings in a tendon. Two nerve-fibres enter into the formation of the same; giving off numerous branches, they finally break up into minute threads, which pierce between the tendon fibrils. The formations have been called **Golgi's bodies** after their discoverer.

7. THE ORGANS OF THE NERVOUS SYSTEM

Fig. 165.—Transverse Section through a Small Branch of the Oculomotor Nerve of the Sheep

280. Fresh frozen section. Hæmalum.

The medullated nerve-fibres, such as we have met before, in the simplest case unite to form small bundles, small **nerves**. To study the structure of such a small nerve, we make thick cross-sections of a fresh ocular muscle with the freezing microtome. The sections are primarily placed in *Ringer's* fluid (p. 25), then stained for fifteen to thirty minutes in hæmalum (p. 56), washed in tap-water, and dehydrated in alcohol. The dehydration must be conducted very cautiously. The sections are fished from the water with a slide, and 50, 70 and finally 95% alcohol is dropped on them; the sections, now hard, are then placed in a vessel containing absolute alcohol. The latter will render them free from water in a few minutes, so that they may be treated in xylol and mounted in Canada balsam.

*Structure of a
Small Nerve Bundle.*

Our figure shows a small nerve. We immediately notice the extremely variable thickness of the single **nerve-fibres**. The **axis-cylinder** appears in each fibre as a deep blue, round, centrally situated, cross-section. In the smallest fibres it may appear as a mere dot. It is surrounded by an unstained or at least very weakly stained ring, the **medullary sheath**. The latter is surrounded by a deep blue circle, in which we frequently see a nucleus, placed like a stone in a signet-ring. This is the **sheath of Schwann** (*sk*) with its nuclei. Between the fibres we also notice nuclei (*ek*), which belong to connective tissue, unstained in our specimen, which fills out all the space between the nerve-fibres and has been termed the **endoneurium**. This endoneurium also forms a separate sheath around each nerve-fibre, the **endoneural sheath**, which is not visible either in our specimen. Going from without inward, we thus have in each peripheral nerve-fibre first the connective tissue endoneural sheath, then the sheath of *Schwann*, followed by the

medullary sheath and finally the axis-cylinder. On the periphery of each such nerve-bundle the endoneurium condenses to form an outer covering, the **perineural sheath** (*ps*). In the latter the connective tissue fibres have arranged themselves in concentric lamellæ and are mixed with elastic fibres. Between the lamellæ we find flat connective tissue cells, which externally form a sort of epithelial covering, separating the nerve-bundle from its surroundings.

PLATE 72

**Fig. 166.—Transverse Section through the Great Sciatic Nerve
of Man**

Fig. 166.—Transverse Section through the Great Sciatic Nerve of Man

60. $\frac{3}{4}$. Formalin-Mueller's fluid. Sodium carminate. Paraffin section. Picroindigocarmin.

A piece of the sciatic nerve, several centimetres in length, is excised from a fresh human body, fastened on a wax plate at both ends under slight tension, and fixed in formalin-Mueller's fluid (p. 34) for twenty-four hours. The next day the specimen is placed in repeatedly changed pure Mueller's fluid, where it remains for ten to fourteen days. After this time the nerve is cut into pieces, 0.5 cm in length; these are washed superficially in water and stained in a 2% solution of sodium carminate. After being rinsed in water the pieces are dehydrated in alcohol, treated in xylol and embedded in paraffin. Paraffin sections, 10–15 μ thick, are counterstained in picroindigocarmin (p. 67) for ten minutes, washed in 70% alcohol, dehydrated and mounted in balsam.

Structure of a Large Nerve.

Our specimen demonstrates how each large nerve consists of larger and smaller nerve-bundles, each of which has the same structure as a small nerve. Our picture shows each bundle surrounded by a green **perineural sheath** (*ps*), containing within the **nerve-fibres**, each of the latter being enclosed in an **endoneurium** (*en*). The fibres in turn appear subdivided into bundles by endoneural septa. The latter bear the larger blood-vessels, giving off branches, which encircle the individual nerve-fibres. The axis-cylinders of the nerve-fibres have been stained red in our specimen by the sodium carminate.

The bundles composing the nerve are surrounded and held together by loose connective tissue, which also surrounds the nerve-trunk on all sides, connecting it to the surrounding tissues. This **epineurium** (*ep*) is mostly rich in fat (*f*) and harbors the large blood-supply (*a* and *v*) of the nerve. In our specimen its purely blue color differentiates it well from the green perineural sheath.



Fig. 166.

PLATE 73

**Fig. 167.—Transverse Section through the Human Cervical
Spinal Cord at the Height of the IV. Cervical Segment**

Fig. 167.—Transverse Section through the Human Cervical Spinal Cord at the Height of the IV. Cervical Segment

12. Formalin-*Mueller's* fluid. Celloidin section. Medullary-sheath-staining.

Technique of the Staining of the Medullary Sheath.

For a general view of the structure of the spinal cord those sections are most suitable in which a distinct color has been imparted to the medullated fibres. In addition to the staining of the medullary sheath we may institute a suitable cell-staining process. The entire, fairly complicated procedure is as follows. The spinal cord from a fresh human body is divided with a sharp razor into segments, each of which includes the origin of a spinal nerve, care being taken not to compress the cord. The sacral portion with the conus medullaris remains intact. The pieces are fixed in 10% formalin for two days, washed in running water for several hours, and transferred to a large quantity of repeatedly changed *Mueller's* fluid (p. 31) for two to three weeks. After washing in running water for one to two hours, we dehydrate in increasing grades of alcohol in the dark. From the absolute alcohol the pieces are transferred, also in the dark, to a mixture of equal parts of absolute alcohol and ether, where they remain for twenty-four hours. From there they go for fourteen days each to celloidin I, celloidin II and celloidin stock solution (p. 46) in well-closed glasses. Now they are mounted on stabilit blocks (p. 47) and kept in 80% alcohol. In making the sections, our aim is not so much to obtain thin, but immaculate sections—15–30 μ is a very suitable thickness for our purpose.

Since the amount of chrome salts in our sections has been materially reduced by the prolonged action of alcohol and celloidin, and as these salts are prerequisites for our success in staining, we transfer the sections from the alcohol first to water and then once more for one to two days to *Mueller's* fluid, to which we add chromic acid at the rate of 1–2 cm³ of a 1% solution to each 100 cm³ of *Mueller's* fluid. The chromic sections are now briefly rinsed in water and placed in a 1% watery solution of hæmatoxylin. This is conducive to the formation of a chrome hæmatoxylin lake within twenty-four hours. After rinsing the black sections briefly in water, we transfer them to a 1% solution of lithium carbonate. The black color will now change to a deep blue, large amounts of color being given off. The lithium solution is changed, until no more color clouds are formed. After rinsing in water we may start to differentiate. For this purpose we use two solutions, a 0.25% solution of potassium permanganate and a thin solution of sulphurous acid, which we can make by mixing equal parts of a 1% potassium sulphite and a 1% oxalic acid solutions. The differentiation takes

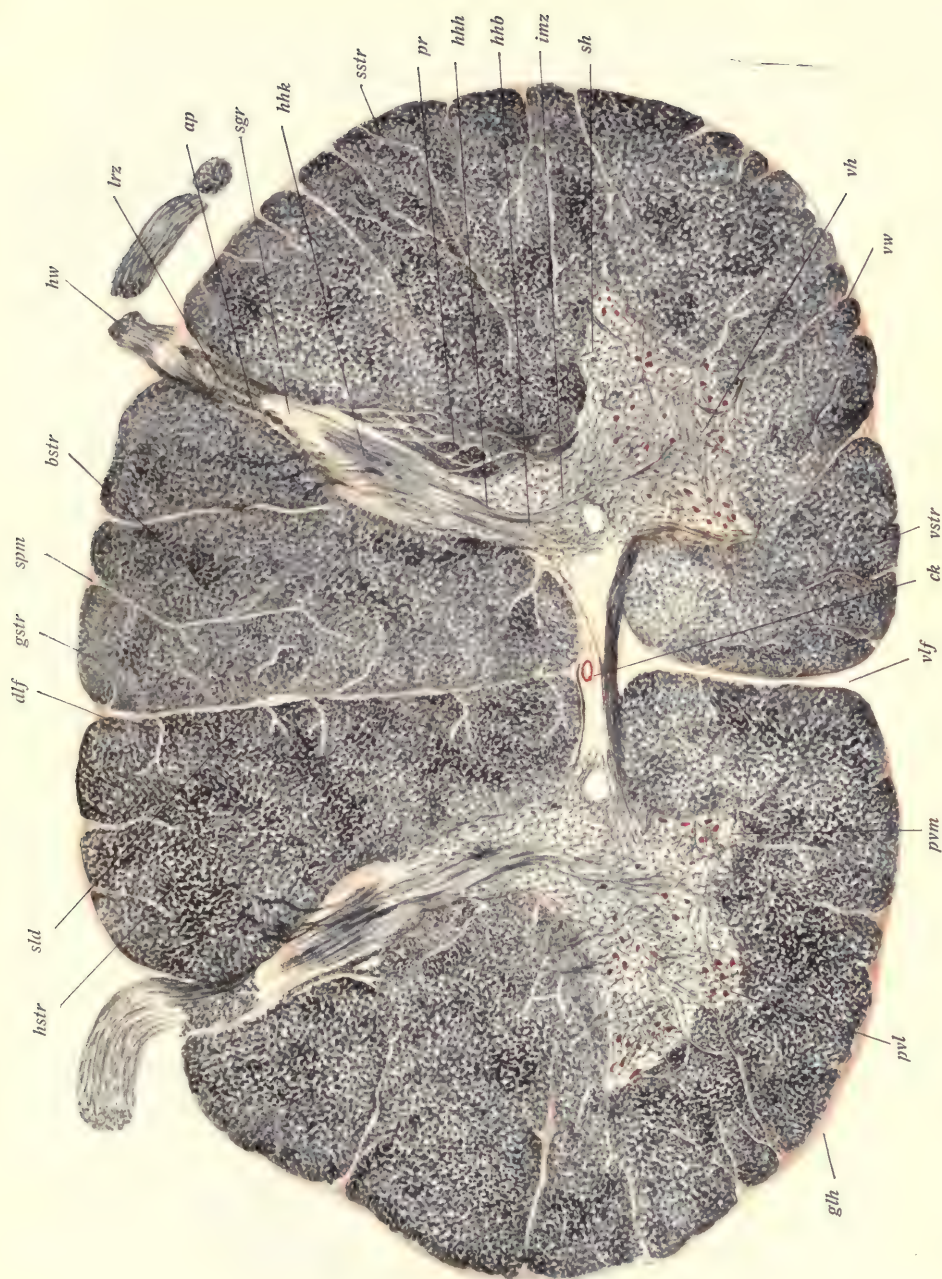


Fig. 167.



place in large watch glasses, three of which are placed in a row. The first contains the permanganate solution, the others are filled with the sulphurous acid. Besides these we have large vessels full of water. With a spatula we place a single section in the first glass for a few seconds, then in the second and, after differentiation has started, in the third. The differentiation is to be discontinued when the gray matter has taken on a light grayish-blue hue, differing sharply from the dark blue medullary coat, and we are able to perceive the almost colorless glia-sheath surrounding the latter in a thin layer. When this effect has been produced we place the section in water; if the latter is too dark, we replace it in the first glass and put it through the differentiation process once more. It is important that after the differentiation the sections should be washed in repeatedly changed water, or, better still, in running water overnight. The following day we stain five to ten minutes in picrocarmin (p. 67), followed by dehydration. The sections only go as far as the 95% alcohol, when they are taken out and placed successively in carbolxylol and xylol (p. 78) and mounted in Canada balsam. In order to have a nicely spread specimen, we place a suitable lead ball on the cover-glass during the first day.

*General View of the Structure
of the Spinal Cord.*

To gain a general view of the structure of the spinal cord, we select a section from the fourth cervical segment, which we study under very low power (objective 1 a). Externally the section is surrounded by the yellowish-red **glia capsule** (*glh*); covering both spinal hemispheres, it enters the **anterior longitudinal fissure**, sulcus longitudinalis ventralis (*vlf*), fills the narrow **posterior longitudinal fissure**, sulcus longitudinalis dorsalis (*dlf*) almost entirely, and from there, as **septum longitudinale dorsale**, divides the two halves of the cord in sagittal direction, so that they are only connected by a small bridge. Furthermore, this capsule sends branching and anastomosing septa into the white matter, dividing its mass of fibres into numerous larger or smaller bundles. Of these septa the **septum paramedianum** (*spm*), located laterally to the posterior longitudinal septum, is of special importance.

The white medullary coat, enclosing the gray matter, thus appears completely divided into two halves. Each of these can be subdivided into three incompletely separated regions, called tracts, an **anterior tract**, a **lateral tract** and a **posterior tract**. The wedge-shaped posterior tract, funiculus posterior (*pster*), is bounded in the median line by the posterior longitudinal septum, laterally by the tapering posterior extremity of the gray matter and the adjoining **posterior root**. The septum paramedianum separates it incompletely in a median division, **Goll's tract**, funiculus gracilis (*gstr*), and a lateral portion, **Burdach's column**, funiculus cuneatus (*bstr*).

In the region of the posterior root, the so-called **Lissauer's marginal tract** (*lrz*), the posterior tract merges into the lateral tract, which occupies the largest part of the white medullary matter. Ventrally it has no definite boundary, but in the region of the **anterior root** (*vw*), composed

of thin bundles, it merges into the anterior tract, which extends to the anterior median fissure.

The gray substance forms with its two halves and the commissure, connecting them, the well-known butterfly-shape. Each half consists of an **anterior horn** (*vh*), a **lateral horn** (*sh*), a **posterior horn** (*hh*) and an **intermediate zone** (*imz*), connecting the two former with the latter. The anterior horn extends into the anterior columns with two processes, the *processus ventralis medialis* and *lateralis* (*pvm* and *pvl*), being continuous laterally with the lateral horn; the latter extends far into the lateral tract. Between lateral and posterior horn the lateral tract crowds against the gray matter, forming the so-called **lateral tract angle**, in which the white substance blends with the gray matter in a manner that the latter becomes arranged in a network, the **processus reticularis** (*pr*). The posterior horn arises with a broad base (*hhh*) from the intermediate zone, becomes narrower for a distance (*hhh*), forming a neck, and finally enlarges to form the head of the posterior horn (*hkk*), which dorsally ends by a rounded extremity. The latter is dorsally enveloped by the **substantia gelatinosa Rolandi** (*sgr*), appearing yellowish-red in our specimen. Farther backward the posterior horn comes to an apex (*ap*) in **Lissauer's tract**, thus it does not reach the periphery of the cord.

The commissure connecting the two halves of the spinal cord encloses the **central canal** (*ck*). It lies slightly nearer to the ventral extremity of the posterior longitudinal septum than to the floor of the anterior fissure, dividing the commissure into a large ventral and a small dorsal half. In each half we can distinguish a portion of gray matter, stained yellow, near the central canal, the *commissura grisea ventralis* and *dorsalis*, and a white portion, stained dark blue, near the floor of the anterior fissure, respectively the posterior longitudinal septum, the *commissura alba ventralis*, respectively *dorsalis*. To the sides of the central canal, where the commissure merges into the intermediate zone, we see two transverse sections of large blood-vessels.

Even with this low power we can discern two groups of cells of the gray matter. The *processus ventralis lateralis* as well as the *processus ventralis medialis* contain a group of large nerve-cells, the **anterior lateral** and the **anterior median group of cells**. Continuous with the latter, toward the commissure and closely approximated to the anterior tract, we find the **posterior median group of cells**. Laterally a **posterior lateral cell-group** brings us to the large cells of the lateral horn. In the interior of the anterior horn we find, aside from numerous scattered cells, the well-defined **central group**. The cells of the posterior horn are too small to appear distinct.

PLATE 74

**Fig. 168.—Transverse Section through the Human Spinal Cord
at the Height of the VIII. Dorsal Segment**

**Fig. 169.—Transverse Section through the Human Spinal Cord
at the Height of the III. Lumbar Segment**

Fig. 168.—Transverse Section through the Human Spinal Cord at the Height of the VIII. Dorsal Segment

12. Formalin. *Mueller's* fluid. Celloidin section. Medullary sheath-staining.

Changes in the Dorsal Cord.

We will now endeavor to study the changes in the spinal cord, taking place at the different heights, selecting first a section of the dorsal cord. Aside from a material reduction in size of the entire cross-section we find several changes in form. While in the preceding section the transverse diameter was considerably larger than the dorso-ventral, we find both to be about equal, so that the previous ellipse has changed to a circle. The most striking change has taken place in the gray matter; it has absolutely and relatively, i.e., in proportion to the white matter, decreased in size and adopted an essentially different form. The lateral horn has noticeably receded, accompanied by a reduction in size of the processus reticularis. The anterior horn has changed its club-shape to a rectangular form, the number of its cells appears diminished. On the other hand, we notice a very distinct round group of cells, located laterally and slightly dorsally of the central canal, called **Clarke's column** (*cls*). It also appears in the cervical cord, but not very markedly. The commissure is short and relatively broad. The posterior horn is extraordinarily slender, its body hardly differing from the neck. The glia or neuroglial capsule of the cord is extremely thin in this section; the sulcus paramedianus of the posterior circumference has disappeared.

Fig. 169.—Transverse Section through the Human Spinal Cord at the Height of the III. Lumbar Segment.

12. Formalin. *Mueller's* fluid. Celloidin section. Medullary sheath-staining.

Changes in the Lumbar Cord.

In the lumbar cord the cross-section approaches the oval form again, the transverse diameter exceeding the dorsoventral. The surface-area of the lumbar enlargement is slightly larger than that of the dorsal cord. The gray matter has become thrice as extensive, so that its proportion to the white matter has gained. If the different segments of the spinal cord are compared in this respect, we notice that the surface area of the white matter remains proportionate to the size of the cross-section, reaching its maximum in the cervical enlargement; later it decreases, first abruptly, then

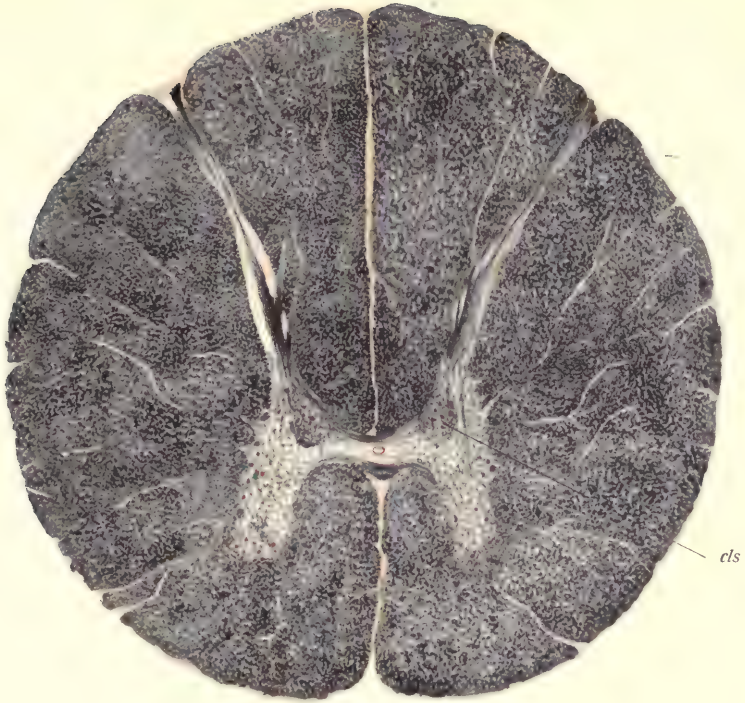


Fig. 168.

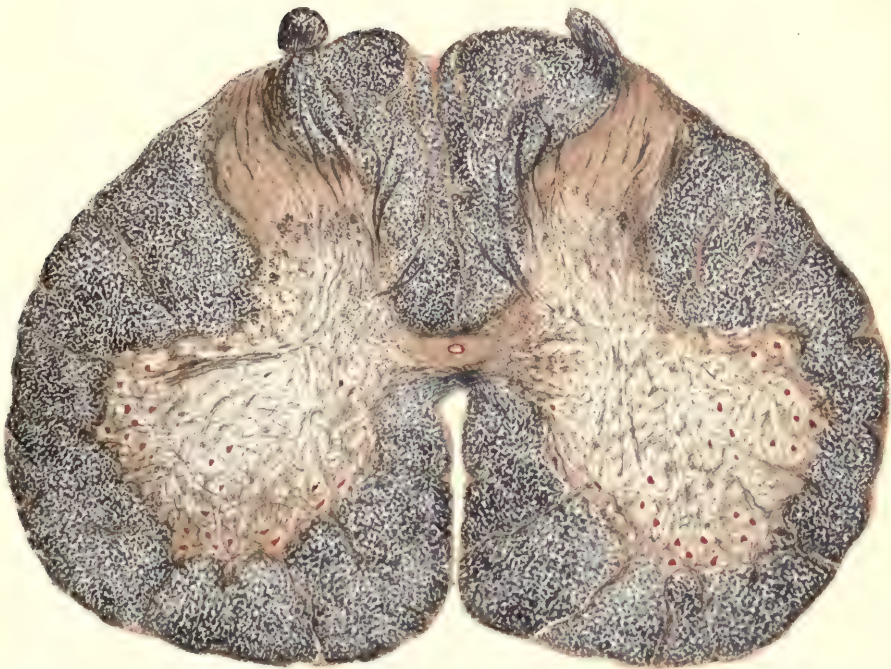


Fig. 169.

gradually up to the end of the dorsal cord; in the lumbar enlargement it increases again, without regaining the size of the cervical enlargement, slowly decreasing once more in the sacral cord. The gray matter acts differently, it increases in the cervical enlargement, soon to decrease again, but thereafter steadily increases in the dorsal and lumbar cord, reaching its greatest dimensions below the lumbar enlargement; in the sacral cord it slowly decreases.

The shape of the anterior horn is more rounded, the processus ventrales are not prominent, the lateral horn is less conspicuous, but the posterior horn is well differentiated, its head is well developed and invaginated by the strong substantia gelatinosa *Rolandi*, which in this region reaches about its maximum, advancing almost to the dorsal circumference of the cord, being separated from it only by a very narrow zone of *Lissauer's* tract.

The various groups of cells of the anterior horn are very distinct; ***Clarke's column*** also is easily recognized, although containing but few cells. In the left half of the cross-section, emerging from *Clarke's* column, we see a bundle of medullated fibres, crossing the intermediate zone and the lateral horn, called the ***bundle of Flechsig***. More or less thick bundles of medullated nerve-fibres radiate into the gray matter from all sides, especially at the posterior horn. Here they enter from the posterior tracts, partially cross the substantia gelatinosa *Rolandi*, and pierce deeply into the anterior horn. Other bundles come from the anterior and lateral tracts and enter the anterior horn.

PLATE 75

Fig. 170.—Transverse Section through the Base of the Human Brain at the Height of the Pyramidal Crossing

Fig. 171.—Transverse Section through the Base of the Human Brain at the Height of the Lemniscal Crossing

Fig. 170.—Transverse Section through the Base of the Human Brain at the Height of the Pyramidal Crossing

12. Formalin. *Mueller's* fluid. Celloidin section. Staining of the medullary sheath.

Pyramidal Crossing.

A few sections through the base of the brain will acquaint us with this exceedingly important region. The technique is the same as that employed in the spinal cord. After removing the hemispheres of the brain and cerebellum, we divide the base of the brain into cross-cuts, 1–2 cm in thickness, which are treated as before mentioned. The cerebral hemispheres and the cerebellum are treated in the same manner.

A section through the pyramidal region shows almost complete crossing. At the left side the place of the former anterior tract has been taken by an extensive area of medullated, transversely cut fibres, the **pyramid** (*py*); at the right the formation of the pyramid has not advanced so far; here we can see the fibres coming down from the crossing point (*dpy*), situated in the depth of the anterior fissure.

Of the remaining parts of our original cross-section of cord we recognize dorsally *Goll's* and *Burdach's* columns, which are sharply defined, but have greatly changed. Large numbers of nerve-cells are seen within their interior, each forming a nucleus, the **nucleus funiculi gracilis** (*nfg*) and the **nucleus funiculus cuneatus** (*nfc*). Ventrally we readily see the head of the posterior horn with its **substantia gelatinosa Rolandi** (*sgr*). It is separated from the periphery by bisected bundles of medullated fibres (*Va*), which represent the **spinal root** of the **trigeminal nerve**, which can be traced far down in the cervical cord. Ventrally of the posterior horn we see a small area of the white matter, the **lateral cerebellar tract** (*ksb*); further ventrally we find **Gower's tract** (*gb*). The former anterior horn is blended with white matter, appears reticulated, and contains but few cells, which belong to the region of origin of the **spinal accessory nerve**.

The **central gray** begins to form around the central canal, two separate masses of cells appearing within it. The dorsal one (*n XI*) represents the **dorsal nucleus of the spinal accessory nerve**, the ventral nucleus of which must be sought in the scattered cells of the anterior horn. The root-bundles, leaving this nucleus, appear at the left side (*XI*); they are already found in the upper cervical cord. The ventral group of cells in the central gray matter (*n XII*) belong to the nucleus of the **hypoglossal nerve**.

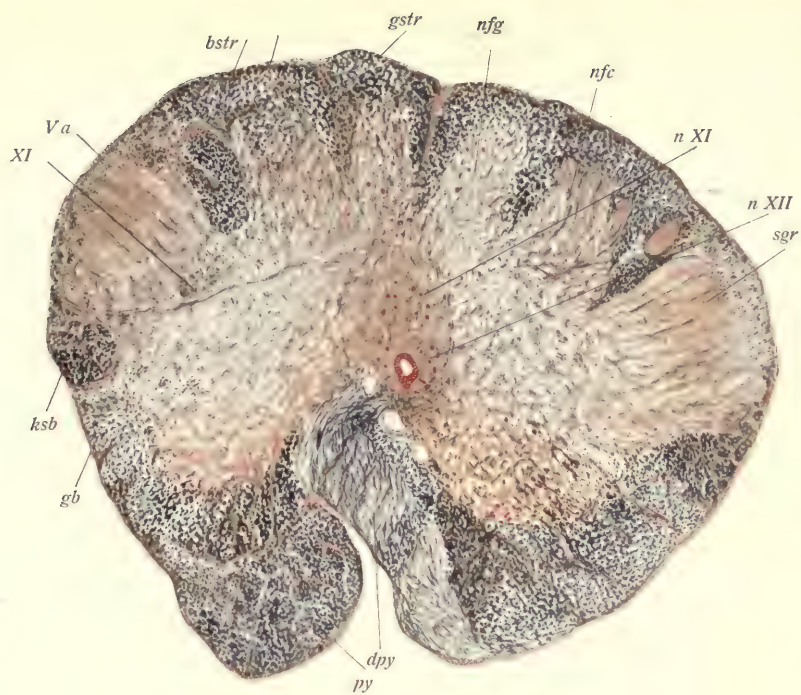


Fig. 170.



Fig. 171.

Fig. 171.—Transverse Section through the Base of the Human Brain at the Height of the Lemniscal Crossing

12. Formalin. *Mueller's* fluid. Celloidin section. Staining of medullary sheaths.

Crossing of the Lemnisci.

The following section demonstrates the completion of the pyramidal crossing and the result thereof, the **pyramids** (*py*), which occupy the greater part of the ventral surface of the section, appearing as two huge triangular bodies. The place of the pyramidal crossing has been taken by the lemniscal crossing (*dl*). The lemniscal fibres emerge from the nuclei of the posterior tracts, curve around the central gray matter, crossing in front of the latter and gather behind the pyramid to form the **lemniscus**.

The reticulation of the region of the former lateral horn is much more advanced, so that we now can speak of a well-formed **substantia reticularis** (*sr*), which laterally and ventrally is continuous with the central gray matter. It contains small, bisected bundles of medullated nerve-fibres, separated by gray substance, containing nerve-cells. Between the substantia reticularis and the dorsal end of the pyramid we notice a small group of cells, the **nucleus of the anterior funiculus** (*nvstr*); they represent the remains of the former anterior horn.

In the remaining parts of our section little has changed; we notice that the central gray matter recedes steadily toward the dorsum, reaching to the floor of the sulcus longitudinalis dorsalis, which dips in deeply at this point.

PLATE 76

**Fig. 172.—Transverse Section through the Base of the Human
Brain at the Height of the Olive**

Fig. 172.—Transverse Section through the Base of the Human Brain at the Height of the Olive

12. Formalin. *Mueller's* fluid. Celloidin section. Staining of medullary sheaths.

The Region of the Olive with the Origin of the Hypoglossal, Vagus and Glossopharyngeal Nerves.

The next sections bring us to the posterior portion of the fourth ventricle. The central canal has widened, allowing the central gray matter to come to the surface, thus imparting a characteristic appearance to the floor of the fourth ventricle. Closely to the median line we again find the **nucleus** of the **hypoglossus** (*n XII*) with numerous nerve-cells, situated with a dense framework of nerves. The formerly dorsally located **nucleus** of the **vagus** (*n X*) lies to the side of the preceding nerve, and still farther laterally and slightly dorsally we find the **acoustic nucleus** (*n VIII*). The floor of the fourth ventricle is lined with ependyma cells; dorsally it is reflected over the **ponticulus**.

The **raphe** has appeared in the median line, dividing the section into a right and left half; it is formed by the crossing nerve-fibres. To the right and left of it spreads the **substantia reticularis**, which presents an inner portion, poor in cellular elements, and an outer, containing a large amount of cells.

The greatest characteristic of our section finds expression in the **olive** (*oi*), that peculiarly shaped leaf of brain-tissue, occupying the greater part of the ventral surface, which, as we all know, attracts attention externally as well. In the median line we find the **pyramid** (*py*), following the olive ventrally. At its ventro-median corner a small collection of nerve-cells appears, the **nucleus arcuatus** (*na*). Immediately dorsal of this we find, on either side of the median line, the **lemniscus** (*lm*). Aside from the large or inferior olive (*oi*), our section shows two small olives; one of these, the **median para-olive** (*om*), lies in the hilum of the olive, the other, the **dorsal para-olive** (*od*), appears lying obliquely across the end of the dorsal surface of the olive. Alongside of this a small collection of cells, the **nucleus ambiguus** (*na*), represents the motor nucleus of the vago-glossopharyngeal nerve. A third origin of these nerves is found in the **fasciculus solitarius** (*IX a*), situated laterally to the sensory nucleus of the vagus. It consists of a bundle of transversely cut medullary fibres, containing some nerve-cells. The fibres, derived from these three nuclei, join at the height of the fasciculus solitarius, leaving the brain in a nearly horizontal course. Our picture only shows their exit (*X*).

Aside from the root-fibres of the vago-glossopharyngeus, our section

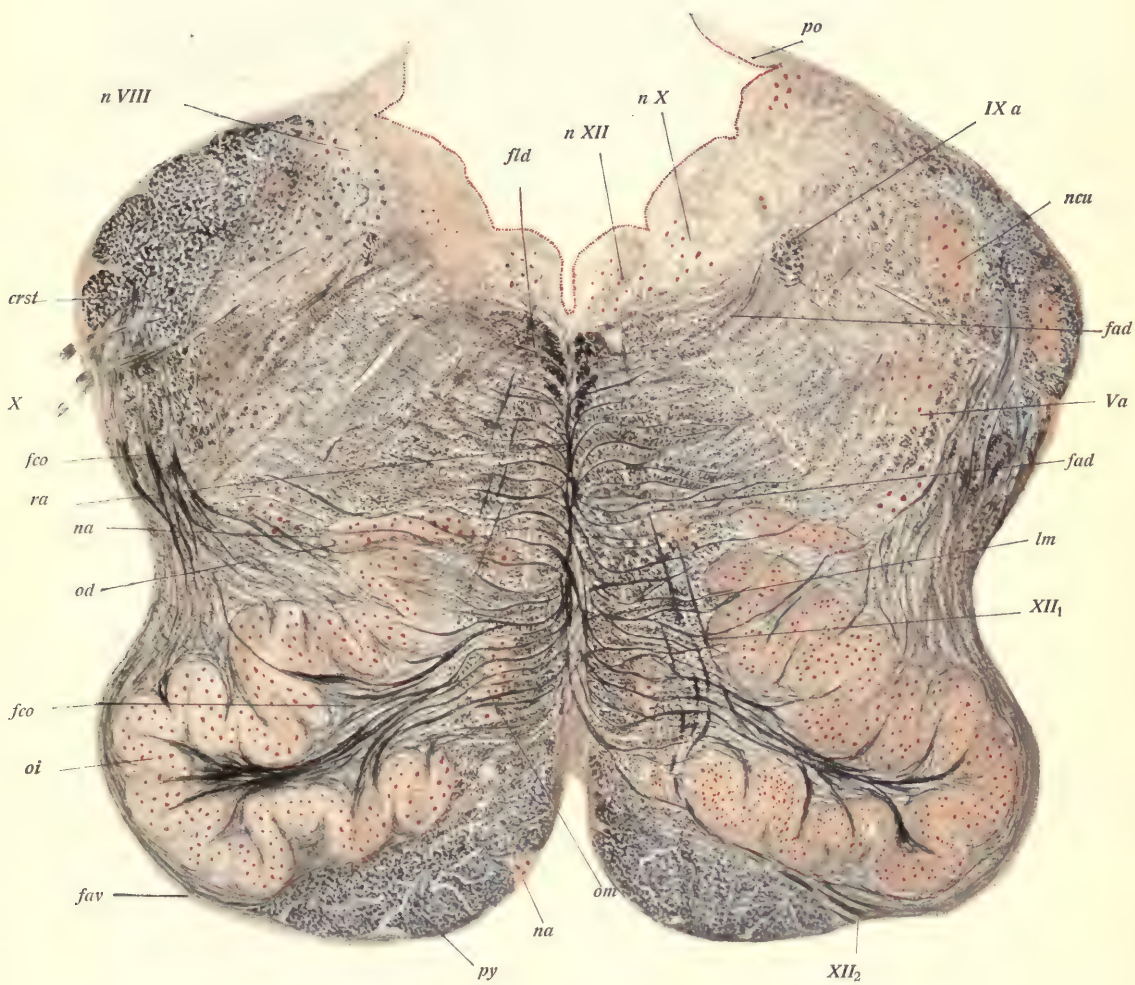


Fig. 172.

also presents those of the hypoglossal nerve. Emerging from the nucleus they travel in an acute angle toward the raphe (*XII₁*), partly encircle the hilus of the olive, partly entering it, traverse the ventral portion of the olivary leaf and emerge between it and the pyramid (*XII₂*).

The space between the olives and the terminal nuclei at the floor of the fourth ventricle is occupied by the **substantia reticularis** and huge masses of medullated fibres. Of the latter we distinguish **horizontal, curved** and **cerebello-olivary fibres**. The horizontal fibres form an important constituent of the **corpus restiforme** (*crst*). They occupy the dorso-lateral corner of the section, comprising the former lateral cerebellar tract and masses of fibres, derived from the nucleus gracilis. Of the curved fibres we differentiate between dorsal and ventral fibres. The former, **fibrae arcuatae dorsales** (*fad*), are derived from the sensory nuclei of the posterior tract; leaving the olive, they describe a curve over to the raphe at the dorsal aspect. They either cross here or traverse the dorsum of the olive, thus reaching the raphe. After reaching the latter, their course at first is either dorsal or ventral for some distance, before turning off to the other cerebral hemisphere. The ventral arcuate fibres, **fibrae arcuatae ventrales**, encircle the ventral surface of the pyramids, emerge from the raphe and enter the arcuate nucleus. Very striking in their course are the **cerebello-olivary fibres** (*fco*); they come from the restiform body, join the arcuate fibres, traverse the olive of the same side and, crossing at the raphe, they enter the hilum of the opposite olive.

Between the olives the raphe is bounded on either side by the **lemniscus** (*lm*); joining the latter dorsally is the **fasciculus longitudinalis dorsalis** (*fld*). On it we find, closely anterior to the nucleus of the hypoglossus, slightly to the right side, a small gray mass, **Roller's nucleus**.

PLATE 77

**Fig. 173.—Section through the Base of the Human Brain at the
Height of the Pons**

Fig. 173.—Section through the Base of the Human Brain at the Height of the Pons

10. $\frac{2}{3}$. Formalin. Mueller's fluid. Celloidin section. Staining of medullary sheath.

*The Region of the Pons with
Origin of the Abducens and
Facialis Nerves.*

Our last section presents the region of the pons, being characterized by the powerful oblique tracts of the **fibres of the pons**. They descend from the partly visible **brachium pontis** (*bpo*) and divide into a superficial **stratum superficiale pontis** (*strs*), a deep **stratum profundum pontis** and a median **stratum complexum**. At first the fibres of the pons push the **pyramids** (*py*) deeply down from their superficial location; on the other hand they (the pyramids) are divided into numerous larger and smaller bundles by the fibres of the stratum complexum. Between the fibres of the pons we notice numerous nests of nerve-cells, the **nuclei of the pons**. The fibres cross in the median line, forming a **raphe**, in which some fibres ascend, the **perpendicular fibres of the pons** (*fppo*). The **restiform body** (*crst*) is likewise pushed down by the arms of the pons; it dips down into the cerebellum. The fibres of the pons divide our section into a ventral and a dorsal portion. The former contains the fibres of the pons proper and has been called the **foot of the pons**; the dorsal portion contains the remainder of the cross-section, termed the **hood**.

In the latter we find, next to the dorsal portion of the stratum profundum pontis, the **lemniscus** (*lm*) on either side of the raphe. It has broadened laterally, presenting a median and a lateral portion. Behind the lemniscus we see, near the median line, the remains of the **substantia reticularis** (*sr*) with its nerve-cells, the **nucleus reticularis tegmenti**. Joining it dorsally is the **fasciculus longitudinalis dorsalis** (*fld*). Laterally and slightly dorsally of the lemniscus we come to the cellular mass of the **superior olive** (*os*). It is pierced by fibres, which are approximated to the lemniscus and are derived from the ventral nucleus of the auditory nerve, which is not included in our section. They descend from the region of the restiform body and either directly enter the upper olive or, crossing first in the raphe, gain the upper olive of the opposite side. This collection of fibres, containing also numerous nerve-cells, we have named the **corpus trapezoides** (*ctr*).

The place of the hypoglossal nerve in the previous section has here been taken by the abducens (*VI*). Its initial fibres leave the nucleus on the floor of the fourth ventricle (*n VI*) and traverse the substantia reticularis almost parallel to the raphe. Further laterally the substantia reticularis

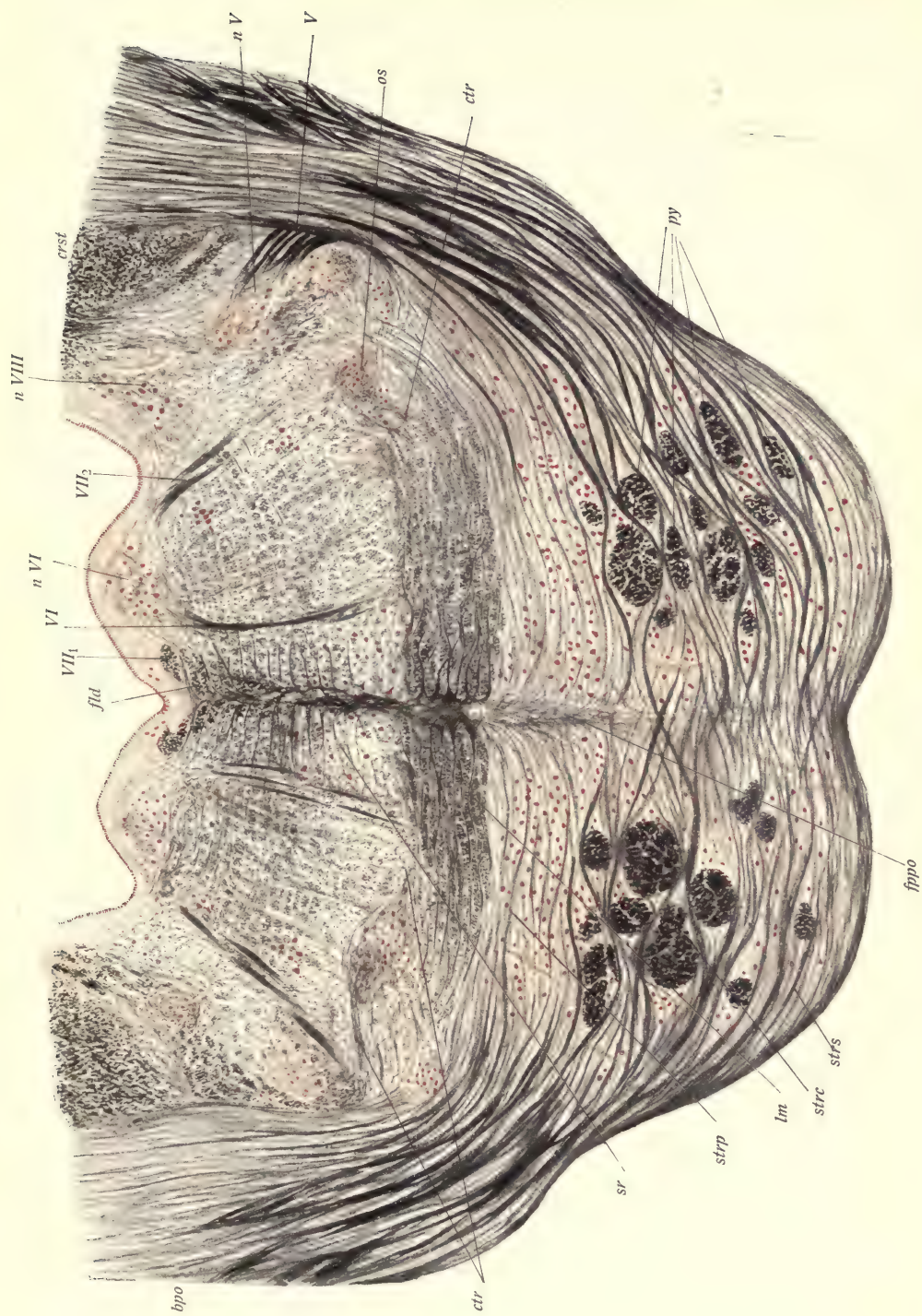


Fig. 173.

is bounded by a second nerve-root, the emerging fibres of the facial nerve (VII_2). The latter seems to arise from the nucleus of the abducens, but in reality only encircles it. Its deep nucleus should be placed in the region of the upper olive of the previous sections. From there the fibres crossed over obliquely, toward the median line and backward, to the inner side of the nucleus of the abducens and thence coursed to the cerebrum in a direction vertical to the transverse section. At this point we can see the nerve in our section (VII_1). We thus have two sections of the facial in our specimen. In subsequent sections the horizontal limb encircles the nucleus of the abducens and then merges into the main egressing limb.

At the side of the exit of the facial we again find the spinal origin of the trigeminus, which at this point enlarges, to form the **sensory nucleus of the trigeminus** ($n V$), which at the right side is joined by the root fibres of the nerve (V). In back of this nucleus lies a small group of large cells, remains of **Deiter's nucleus** ($n VIII$), which belongs to the region of origin of the acoustic nerve.

PLATE 78

Fig. 174.—Cortex of Human Cerebellum

**Fig. 175.—Section through the Cortex of Human Cerebellum
(Temporal Lobe)**

Fig. 174.—Cortex of Human Cerebellum

50. $\frac{2}{3}$. Formalin. *Mueller's* fluid. Celloidin section. Staining of medullary sheaths.

Ascending in the central organ, we reach the cerebellum, a median sagittal section of which will furnish the best pictures. It demonstrates the well-known arbor vitæ, a small branch of which is reproduced in our picture.

Classification of the Cerebellar Cortex.

While in the spinal cord the white substance enveloped the gray like a mantle, we find the reverse to be true here, the axis of each cerebellar leaf consisting of white substance, surrounded by a thick layer of gray matter. Thus we have in the cerebellum an inner **medullary substance** (*mas*) and an outer **cortical substance** (*rs*). In the latter we differentiate three zones. The medullary layer is followed by quite a large zone, distinguished by numerous small cells, the **granular layer** (*ks*). Externally this is surrounded by a single row of extraordinarily characteristic large cells, the well-known **Purkinje's cells** (*ps*). The outermost stratum of the cortex is formed by the **molecular layer** (*mos*), in which a few scattered, small and medium-sized cells have taken the stain.

Medullary Fibres of the Cerebellar Cortex.

The medullated fibres, composing the medullary layer, radiate into the cortex, either in straight course or turning off in a more or less obtuse angle. They are derived mainly from the three cerebellar peduncles, the crura pontis, the corpora quadrigemina and the restiform bodies, through which they leave and enter the cerebellum respectively. In addition to these we have the so-called **associating fibres**, which connect the different portions of the cortex.

We can readily trace these fibres in their course through the granular layer. They partly cross it in a straight course, partly form a reticulum within it, which becomes quite dense below the cells of *Purkinje*, the fibres at the same time changing their course and appearing in cross-section. Enormous numbers of fibres emerge from this plexus, becoming lost between the bodies and processes of *Purkinje's* cells, generally appearing in cross-section. Thus we see that the outer, by far larger, portion of the molecular layer is almost devoid of medullated fibres. About the origin and destination of these fibres we will learn more in a subsequent specimen (p. II, 327).

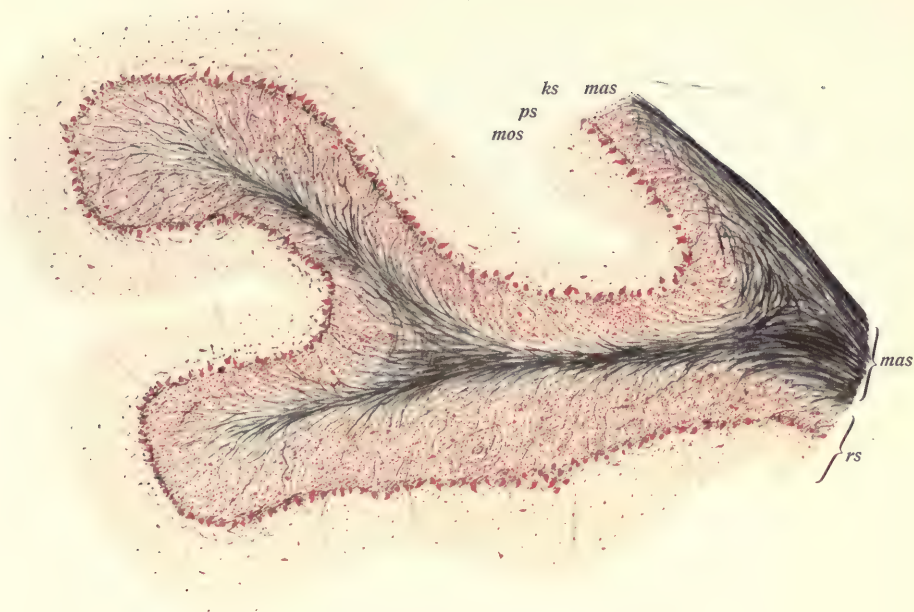


Fig. 174.

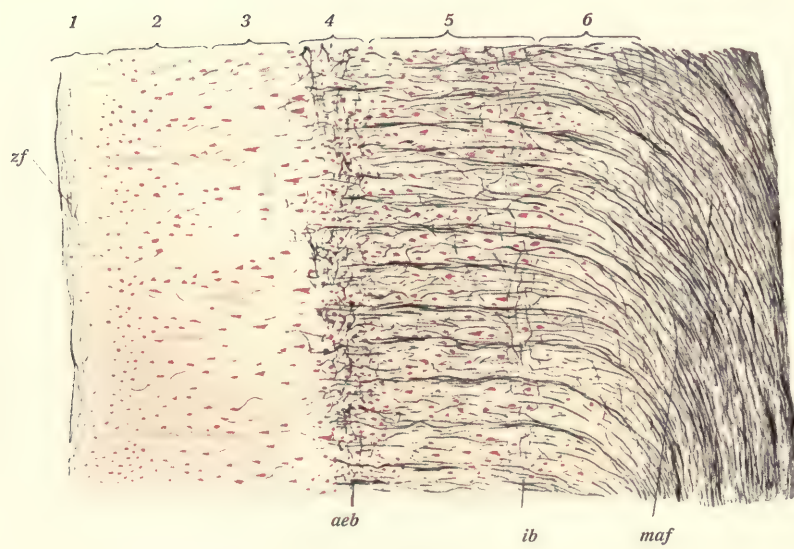


Fig. 175.

Fig. 175.—Section through the Cortex of Human Cerebrum (Temporal Lobe)

50. $\frac{2}{3}$. Formalin. *Mueller's* fluid. Celloidin section. Staining of the medullary sheath.

In the cortex of the cerebrum, which we will now discuss, the arrangement of the medullary fibres and of the cellular element in the various parts of the hemispheres differs widely. We will select a section of the temporal lobe, because the arrangement of the fibres there is especially instructive. It is well for the beginner to select other cortical sections for comparison.

Division of the Cerebral Cortex.

Very similar to our findings in the cerebellum, we notice in the cross-section of each cerebral convolution a central layer of medullated fibres, which is entirely enclosed by **cortical substance**. The fibers composing the **medullary stratum** are partly neurites of cortical cells, leaving one to enter the other; they either leave the cerebellum, **projection fibres**, or go to other parts of the same or the opposite hemisphere, **association fibres**. Besides these we have **centripetal fibres**, which leave the medullary substance and enter the cerebral cortex.

Turning first to the divisions of the cerebral cortex, which can readily be identified in our specimen by the carmine stain of the cells, we find the cerebral cortex is decidedly more complicated than the cerebellar, the division of the different layers being not as definite as it was there. The strata are characterized by the shape of their cells, which we will discuss in detail later on. At the surface of the convolution we have primarily a **molecular layer** (1), poorly equipped with cells. It is followed by two layers of pyramidal cells; at first the cells are small, the **layer of small pyramidal cells** (2), then they become larger, the **layer of medium-sized pyramidal cells** (3). We now come to a stratum of small cells, the **granular layer** (4); it is but very narrow, and in many places of the cortex ill-developed. This stratum is succeeded by the **layer of giant pyramids** (5), which, aside from smaller elements, contains those mighty, large giant pyramids which are so characteristic of the cerebral cortex. Finally, we have a last layer, next to the medullary substance, the **layer of polymorphous cells** (6), characterized by smaller cells, often of spindle shape.

Medullary Fibres of the Cerebral Cortex.

Let us proceed to the medullary fibres. Curving away from the medullary substance, they radiate into the cortex, where they form parallel bundles, the **medullary fibres**. The bundles become thinner and thinner, until they are lost in the third layer. They consist largely of the neurites of the pyramidal cells.

Aside from the **radiating fibres**, the cortex contains an endless number

of tangential fibres. Primarily we notice closely under the surface of the cortex a layer of fibres, which are partly in longitudinal, partly in oblique, partly in transverse section. They are the **zonal fibres** (*zf*). Their course is partly parallel to the longitudinal axis of the convolutions, partly at right angles to the latter, viz., parallel to the surface of the convolutions. In the middle and deeper parts of the cortex we also notice three other strands of tangential fibres, one at about the height of the granular layer, the **outer fascicle of Baillarger** (*aeb*), another within the layer of the giant pyramids, the **inner fascicle of Baillarger** (*ib*), and a third in the deepest strata of the cortex, the **associating fibres of Meynert** (*maf*). Between these various fascicles we also find a fine reticulum of medullated fibres, which extends into the layer of the small pyramidal cells, but is scarcely demonstrated here, owing to the low power used.

PLATE 79

Fig. 176.—Lumbar Cord of the Child

Fig. 176.—Lumbar Cord of the Child

20. Rapid chrome-silver method.

For the demonstration of the cells of the spinal gray matter we use a so-called *Golgi* specimen of the cord of a child. The process has previously (p. 70) been exhaustively described. The best pictures are always obtained from the upper and middle portion of the lumbar cord. We will select such a section for our description.

Fig. 176 shows a section such as we seldom obtain. It is not a combination picture, differing from a true natural reproduction only inasmuch as a large amount of cells, found in the specimen, have not been represented.

In the anterior horn we see depicted a number of **motor root cells** (*vroz*). We are familiar with their shape from previous study (pp. II, 115, 118, 121). The numerous dendrites can be traced for long distances in the gray matter, and are also seen piercing more or less into the white matter. Aside from these anterior root-cells we also find some cells in the median portion of the anterior horn, which are called **commissural cells** (*coz*), after the nature of their neurites. In shape they either resemble the preceding or appear more drawn out and, keeping close to the white matter of the anterior tract, they pierce into the anterior commissure. Their neurite traverses the latter and, reaching the anterior column of the other side, becomes an anterior column fibre. A third form of cells, the **columnar fibres** (*strz*), is found in almost every portion of the gray matter, but especially in the middle portions of the anterior horn, in the lateral horn and in the posterior horn. The neurite of each of these cells, after taking on a medullary sheath, pierces into the white matter, where it divides into an ascending and a descending branch. These cells are very numerous, their neurites thus forming an important constituent of the white matter of the spinal cord. For example, we know the ground bundle of the antero-lateral tract to consist mainly of the neurites of the columnar cells of the anterior and lateral horns, the neurites of the columnar cells of the posterior horn coursing in *Burdach's* and *Gower's* tracts.

A special variety of columnar cells presents itself in **Clarke's column** (*clz*). These cells are very well demonstrated in our specimen. They are large cells, with numerous short, gnarled dendrites. The neurites emerge from the ventral circumference of the column in form of strong bundles (*clnt*), traverse the posterior horn obliquely and enter the white substance of the lateral tract, where they form the lateral cerebellar tract.

Only a few of the cells of the posterior horn have been impregnated. They are mostly small, elongated cells (*hhz*), sending off neurites to the lateral and posterior columns. The **border cells** (*grz*) are slightly larger, situated at the dorsal border of *Rolando's* substance; they run obliquely to the axis of

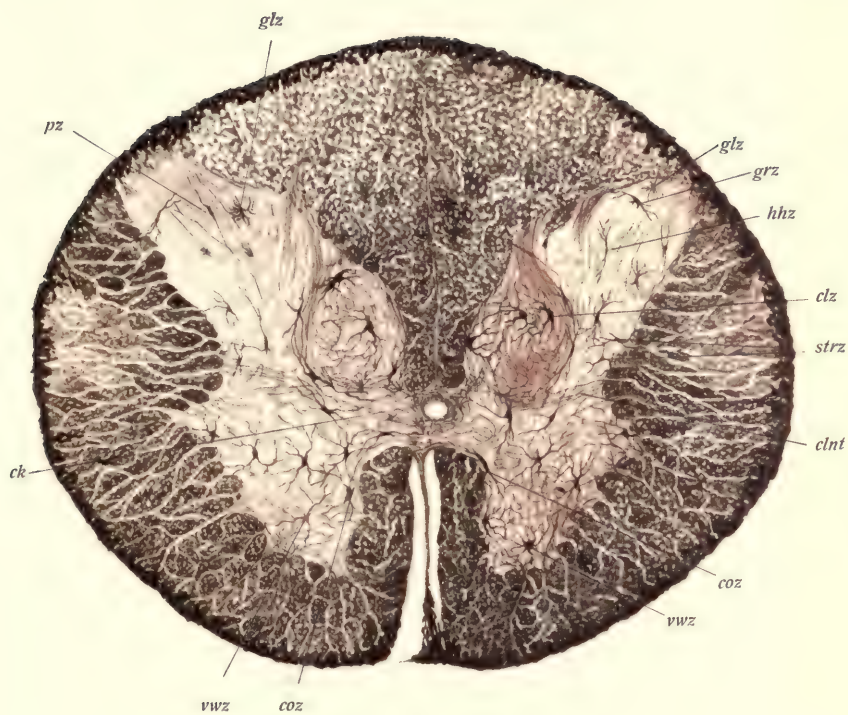


Fig. 176.

the posterior horn, sending their neurites into the lateral horn. Derived from the same substance, we find at the left side of the specimen a **pyramidal cell** (*pz*). The neurite, arising from the dorsal extremity of the cell, turns toward the posterior tract.

Of the **glia cells** (*glz*), which are present in large numbers, only a few have been represented. The small cell-body sends minute processes in all directions. These **astrocytes** are especially numerous around the central canal (*ck*), where they form a thick neuroglial ring, the **substantia gliosa centralis**. Many specimens will also show the impregnation of several ependyma cells.

PLATE 80

Fig. 177.—Cervical Cord of the Cat

Fig. 177.—Cervical Cord of the Cat

400. $\frac{2}{3}$. Sublimate-nitric acid. Formalin. Frozen section. *Gram's* solution-gold chloride-resorcin.

An additional specimen will acquaint us with the central canal and its environment, giving us also an opportunity to study the structure of the neuroglia. Pieces, 3–5 mm in thickness, of the spinal cord of a freshly killed mammal are fixed in sublimate-nitric acid (p. 31) for five hours, washed in running water overnight and transferred to 5% formalin. Frozen sections, made from these pieces, are treated with gold chloride—*Gram's* solution-method (p. 73). They are mounted in Canada balsam or, better still, in levulose.

Sections, gilded in this manner, furnish excellent demonstrations of the structure of the spinal cord. The axis-cylinders of the medullated nerve-fibres appear deep purplish red, while the medullary sheaths remain unstained or at least are only very light red. The purple-red color is also taken up by the axis-cylinders of the gray matter and the nerve-cells, where the chromatic substance and the structure of the nucleus is beautifully illustrated. The same can be said of the glia cells and the ependyma cells of the central canal. The glia fibres, on the other hand, are deep black, contrasting well from the purple red of the cells and the axis-cylinders. Of course, these fibres can be demonstrated more completely by a specific method, which, however, is much more cumbersome. The red blood corpuscles stain the deepest, thus bringing out the blood-vessels, as far as they are filled with blood. An absolute prerequisite for this method is that the material be absolutely fresh.

Our specimen shows the central canal (*ck*) with the two commissures (*vk* and *hco*) and the adjacent portions of white matter (*vstr* and *hstr*). This picture illustrates the aforementioned fact, that the anterior columns (*vstr*) contain much thicker nerve-fibres than the posterior (*hstr*), and that this change in calibre affects the axis-cylinders as much as the medullary sheaths.

Epithelium of the Central Canal.

The central canal (*ck*), which occupies the centre of our specimen, is lined by a simple layer of cells, which in general may be called cylindric. These ependyma cells are mounted by a broad base on the lumen of the canal; they taper and end in a fine point in the surrounding substantia gliosa centralis. The cells are the longest in the lateral parts, being shorter in the ventral and dorsal aspect of the canal; in the former they appear as short stubs. The free surface, bounding the lumen of the canal, is studded with shorter or longer cilia, but this ciliation is incomplete, probably due to faulty preservation.



Fig. 177.

Substantia Gliosa Centralis.

The substantia gliosa centralis consists of a dense layer of *glia fibres*, which run in three main directions. One portion of the fibres surrounds the central canal in ring-shape, another portion runs parallel to the direction of the canal, therefore appearing in cross-section, and a third and last portion radiates from the central canal. These last fibres appear especially distinct in the anterior commissure, crossing the same in the form of a thick, loose bundle; they can be traced to the floor of the anterior commissure. We call this bundle the **anterior ependyma wedge** (*vepk*). A **posterior ependyma wedge** (*hepk*) is less distinct in our specimen. Here, in the posterior commissure, the fibres converge from all sides, enter the cleft between the posterior tracts of either side, and form the **septum longitudinale dorsale** (*dls*).

Glia Cells and Glia Fibres.

Besides fibres, the substantia gliosa centralis also has a moderate amount of **glia cells** (*glz*). They are small, sometimes medium-sized cell-bodies, drawn out in two or more short processes. They are generally mononuclear, but quite often contain two nuclei. The nuclei, though larger than the nuclei of the ependyma cells, are always considerably smaller than those of the nerve-cells, and are richer in chromatin than the latter.

Studying these glia cells a little closer, we notice that the glia fibres run through the body of the glia cells. We thus have similar conditions, as we found among the cells and fibres of reticulated tissue; we are now able to better understand the pictures of the glia cells, furnished by the chrome-silver method (p. II, 319). There we found cell-body and glia fibres evenly black, the result being apparently cells with numerous long, thin processes. Our gold method, however, shows fibres and cell-body as distinctly separate formations. If we study our ependyma cells in this respect, we find similar conditions. The radiating glia fibres there, also, often pierce to the immediate vicinity of the lumen of the canal, being closely approximated to the bodies of the ependyma cells. Glia cells and ependyma cells are related elements of equal value, both produce glia fibres; their history of development teaches us that the glia cells are but transferred ependyma cells.

Relation Between Glia Fibres to Nerve-Cells and Blood-Vessels.

Our section also shows two nerve-cells (*nz*). They are surrounded by glia fibres, which envelop them like baskets. Small glia cells can be found closely approximated to the bodies of large nerve-cells.

In the anterior commissure we also find a small artery, a central artery (*a*), showing the origin of a small side branch. The glia fibres approach the blood-vessel, turn and form a complete *glioma sheath* around it. Between the sheath and the vessel-wall a narrow perivascular *lymph-space* remains.

In the white matter we likewise find numerous glia fibres and glia cells. The former partly run in a tortuous course between the nerve-fibres, at right angles to their course, partly appear in cross-section, viz., have the same direction as the nerve-fibres. The glia cells show very similar conditions to those of the gray matter.

PLATE 81

Fig. 178.—Cervical Cord of the Cat

Fig. 179.—Cerebellum of the Cat

Fig. 178.—Cervical Cord of the Cat

50. $\frac{3}{4}$. Vital methylene blue staining.

To supplement and widen our knowledge of the structure of the spinal cord we will resort to the vital methylene blue staining method. True, we do not always have success with it, but if we do, it furnishes better results even than the chrome-silver method, and, which is a practical point of value not to be underestimated, it gives us an abundance of material, which is readily preserved. Cat and rabbit are best suited for the process, which is exhaustively described on pages 59–62.

Fig. 178 represents a section from a methylene blue specimen of the cervical cord of the cat. We recognize all the cell-forms found in the child, e.g., root-cells (*vwz*), columnar cells (*strz*), commissural cells (*coz*). The cells, however, are mostly much more numerous and more completely stained with all their processes, dendrites and neurites. Especially the latter can be traced for long distances in the sections of 50–100 μ thickness.

Anterior Root-Fibres.

For example, we can very prettily see the composition of the anterior roots and the origin of their fibres. The anterior roots (*vw*) emerge on either side in three to four bundles from the gray matter, each of which consists of a large number of medullated fibres. Neurites of all sorts of cell-groups of the anterior horn enter into the formation of these bundles, and it is due to this fact that the neurites, in order to arrive from their place of origin to the root-bundles, must often describe a large curve and, secondly, must frequently cross one another. If we look at these neurites with high power, we find the following state of affairs. They arise from the cone of origin of the cell as very thin threads, from one or two loop-shaped curves, and soon swell to three or four times their original thickness. Where this thickening begins, the axis-cylinder takes on a medullary sheath, thus becoming a central, medullated nerve-fibre. This runs, either in a straight course or curved, in a tortuous course, toward its root-bundle and, before leaving the gray matter, at the point of *Ranvier's* constriction, gives off one or more fine side-branches. These *motor collaterals*, which can just about be recognized with our low power, soon split up into innumerable delicate fibrils, which end at the neighboring root-cells.

The Collaterals of the White Matter.

Our specimen also gives us excellent information about the remaining nerve-fibres of the gray matter. Besides the neurites, leaving the gray matter, we find numerous medullated fibres entering from the white into the gray matter, where they terminate. These *collaterals* are seen emerging everywhere from

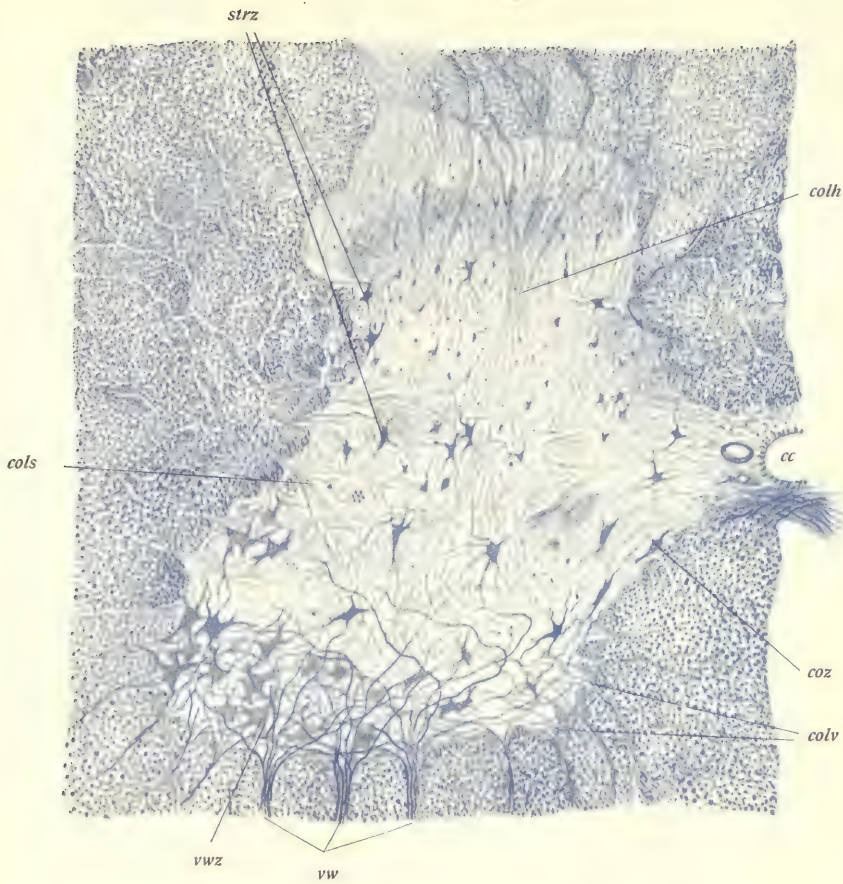


Fig. 178.

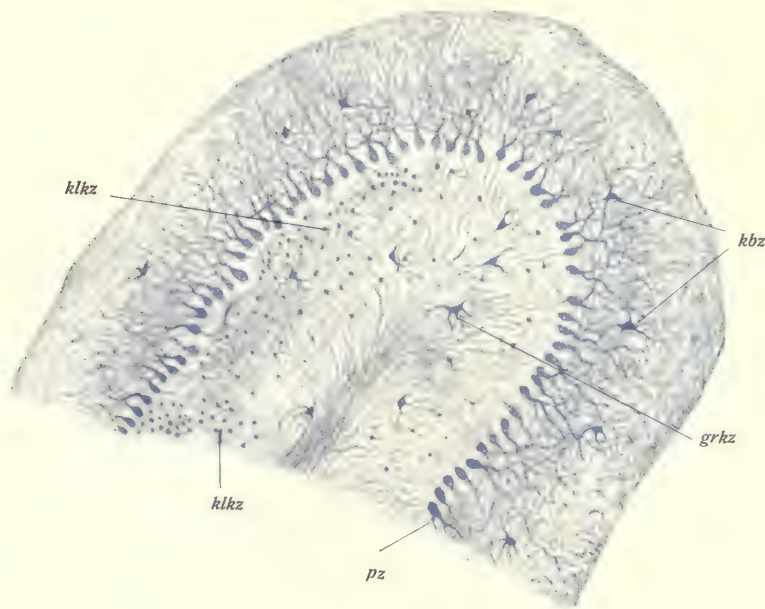


Fig. 179.

the white matter and entering the gray. The **collaterals of the posterior columns** (*colh*) are the most characteristic. They cross *Rolando's* substance and the head of the posterior horn in small, often parallel bundles, ending partly at the cells of the posterior horn and the column of *Clarke*, partly joining to form a thick fascicle, the so-called **reflex bundle**. These fibres pierce through the neck of the posterior horn into the anterior horn, where they spread fanlike and terminate at the cells of the anterior horn. The **collaterals of the anterior tract** (*colv*) are, as a whole, poorly developed, ending, as a rule, at the anterior root-cells. On the other hand, we have very numerous **collaterals of the lateral tract**, which pierce almost all the regions of the gray matter, ending at the cells of the latter. About the details of their termination we can learn so much from high power observation, that the fibres, after losing their medullary sheath, divide into very minute fibrils, which are studded with varicosities and are intimately approximated to the body of the nerve-cells.

About the origin of the collaterals we should consult frontal sections through the cord. Such sections show how the medullated fibres of the white matter, which are derived either from the spinal ganglia, the gray matter of the cord, or from higher portions of the central nervous system, send branches into the gray matter at right angles, which represent our so-called collaterals.

Fig. 179.—Cerebellum of the Cat

60. $\frac{3}{4}$. Vital methylene blue staining.

The vital methylene blue method has likewise advantages for the study of the cerebellum. The process is the same. The sections are placed vertically to the longitudinal axis of the convolutions.

Cells of the Cerebellar Cortex.

The most prominent features are, of course, the cells of *Purkinje* (*pz*). We see them in a simple layer at the boundary between granular and molecular layer. The pyriform cell-body tapers toward the periphery, forming two to three huge dendrites, the branches of which, like a forest filled with trees, penetrate the entire molecular layer. The easily recognized dendrite leaves the blunt end of the cell, takes on a medullary sheath and becomes a fibre of the medullary substance.

The molecular layer appears relatively poor in nerve-cells. We find in it only medium-sized multipolar nerve-cells, the so-called **basket cells** (*kbz*). They are found in the middle and the deeper strata of the molecular layer; they give off one neurite, which runs above the cells of *Purkinje* for some distance, sending a collateral branch to each of them, which surrounds their body with a dense fibrous net.

A considerably larger amount of cells is found in the granular layer. Above all we find here the characteristic **small granular cells** (*klkz*), small, angular cell-bodies, which send off three to four short, strong dendrites, which soon terminate in several short, clawlike terminal branches. Each such claw

grasps the body of a neighboring cell. The neurite of the granular cell is difficult to recognize in our specimen. It is very thin, extending in a radiating course into the molecular layer, where it branches in the shape of a T. The branches are cut transversely in our specimen, since they run in the direction of the convolution and parallel to the surface. Aside from the small granular cells, we also find the **large granular cells** (*grkz*) in the granular stratum. These also ascend into the molecular layer. They are large multipolar cells, giving off numerous dendrites, which are of interest inasmuch as their neurite soon breaks up in a dense texture of fibrils (cells of the II. type of *Golgi*).

We must still mention some fibres which, leaving the medullary substance, enter the cortex and ascend upon the cells of *Purkinje*, earning the name of *climbing fibres*. Our specimen only suggests them.

PLATE 82

Fig. 180.—Cerebral Cortex of Child

Fig. 180.—Cerebral Cortex of Child

60. $\frac{3}{4}$. Chrome silver method.

For the demonstration of the structure of the cerebral cortex the chrome silver method (p. 69) is superior to the vital methylene blue staining. We use the brain of children during the first weeks of life, observing the rules given before.

The infantile cortex does not show such typical arrangement of layers as we met in the temporal lobe of the adult (p. II, 315); still, it demonstrates the various types of cells. Most striking are the large **pyramidal cells** (*grpz*). They are found throughout the middle region of the cortex. As their name indicates, they are large pyramid-shaped cells. Their base points toward the medullary portion, their apex vertically toward the brain surface, giving off numerous dendrites and soon breaking up into two or more branches, trunk dendrites. These, branching constantly, strive toward the cortical surface, where they split up into minute fibrils, running parallel to the surface. The cell-base gives rise to numerous dendrites and the neurite. The latter can easily be traced, especially in thick sections; descending through the deeper portions of the cortex, it enters the medullary matter. During this course it acquires a medullary sheath. The neurites of neighboring cells join to form bundles, which later constitute the characteristic medullary rays (p. II, 315) of the cerebral cortex.

The **small pyramidal cells** (*klpz*) are in all details identical to the large, but, as their name implies, are smaller.

The molecular layer contains few cells. Our specimen shows but one impregnated cell, a small cell-body, sending processes to both sides. These cells are generally designated as **Cajal's cells** (*cajz*). They do not show any neurite, all processes having the character of dendrites. This condition we will again meet in certain cells of the retina (p. II, 355).

In the deeper layers of the cortex the nerve-cells lose their typical pyramidal shape, becoming elongated, triangular or irregular. They are therefore, called **polymorphous cells** (*polz*). Dendrites emanate from them in all directions. The neurite generally behaves similar to that of the pyramidal cells. We find, however, cells the neurite of which does not enter the medullary substance, but, reversing its course, gains the surface of the cortex, where its branches leave at right angles, forming a rich network in the molecular layer.

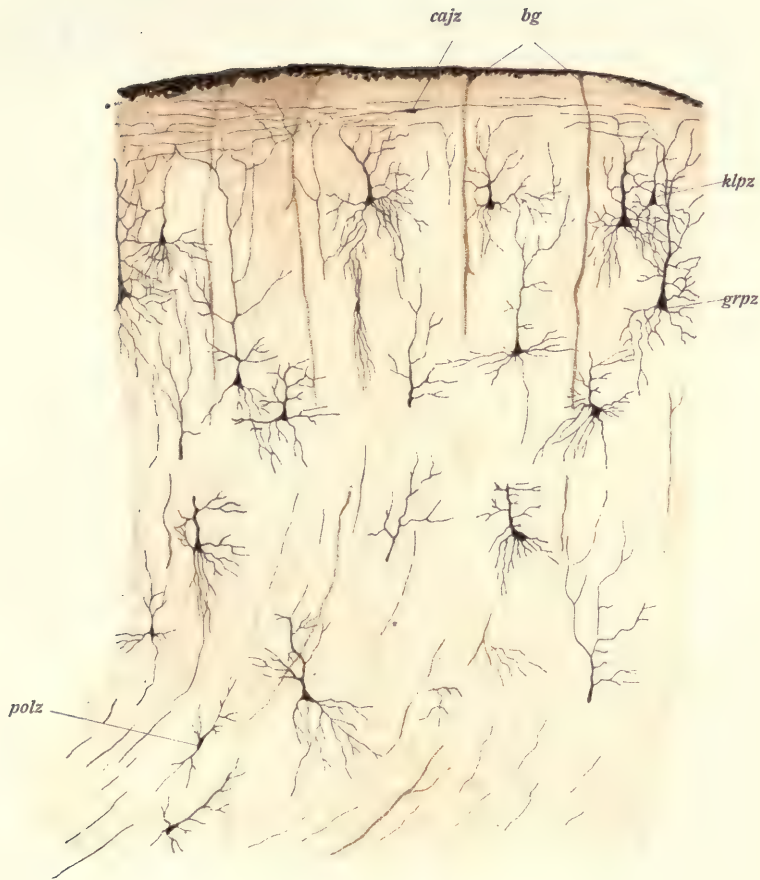


Fig. 180.

PLATE 83

Fig. 181.—Cervical Cord and Spinal Ganglion of the Cat

Fig. 182.—Nerve-Cells from a Spinal Ganglion of the Cat

Fig. 181.—Cervical Cord and Spinal Ganglion of the Cat

15. $\frac{3}{4}$. Vital methylene blue staining.

For the detailed study of the spinal ganglion we use the material of the cat, prepared in the manner related on p. II, 326. If we desire to demonstrate the junction of ganglion and cord, the dura remains unopened. The cord, together with all its ganglia, is taken out and divided into different segments, each of which includes on either side a spinal ganglion with its two roots. For our purpose we select the ganglia of the cervical cord with its short, strong roots. Each segment is first placed on a small wax plate; the spinal nerve of either side is fixed with porcupine bristles under light tension in a manner that the ganglion lies in the centre of the section. The specimen is fixed on the wax plate, washed, dehydrated and only detached in the absolute alcohol. Paraffin sections should be 50–100 μ thick and be mounted preferably in oil of cloves—collodium (p. 46).

Junction of Spinal Ganglion and Cord.

The right half of a section is shown in our picture. The spinal ganglion is seen as an elongated, oval body alongside the spinal cord; it is enclosed in a connective tissue capsule, which is continued on one side around the spinal nerve, on the other over the two roots (*vw* and *hw*) and the dura mater of the cord. The ganglion cells form a layer around the ganglion, and in the interior are segregated into numerous nests by the efferent and afferent nerve-fibres.

The strong posterior root arises from the median aspect of the ganglion, curves over to the dorsal circumference of the cord and enters the posterior tract thereof. The **spinal nerve** (*spn*) enters the lateral portion of the ganglion, but only in part, the other part of its fibres consisting of the fibres of the anterior root, emerging from the cord. While the posterior root enters the posterior tracts in a compact strand, the anterior roots, as we had occasion to notice before (p. II, 326), leave the anterior horn in several bundles, pierce the white matter of the antero-lateral tract, and join within the spinal canal to form an anterior root-bundle, several of which arise above one another and join; this trunk unites, at the outer side of the ganglion, with the sensory portion of fibres, which enter the ganglion; the combination of both portions constitutes a spinal nerve. The anterior roots thus do not come in direct contact with the spinal ganglion.



Fig. 181.

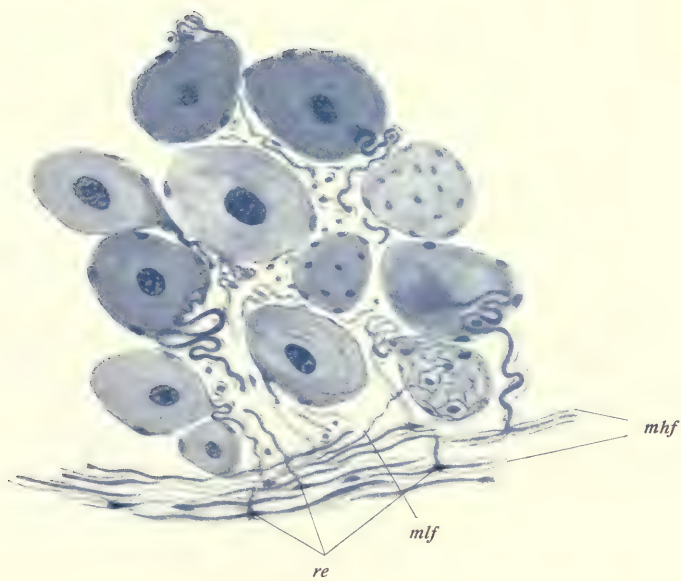


Fig. 182.

Fig. 182.—Nerve-Cells from a Spinal Ganglion of the Cat

280. $\frac{3}{4}$. Vital methylene blue staining.

Finer Structure of the Spinal Ganglion.

Although we were acquainted with the spinal ganglionic cell during our general study of the nerve-cell, it will be of advantage to us to again study these cells here, under high power. We recognize the large, rounded or pyriform cell-bodies, which are either surrounded or covered by their respective capsular cells, according to whether the nerve-cell has been cut right through or only nipped.

In many cells we may perceive the origin of the cellular process with its previously described (p. II, 114) glomerulus formations; we are now also able to follow the course of this process for a long distance. Shortly after leaving the cell, the process takes on a medullary sheath, winds through the mass of neighboring cells and enters a bundle of nerves, which separates the nests of cells, generally at right angles to the course of the fibres. Arrived here, it undergoes a T-shaped bifurcation and merges into a medullated nerve-fibre (*mhf*); thus one portion of the cellular process enters the posterior spinal tract with the posterior root, becoming a posterior tract fibre, while the other portion constitutes a spinal nerve-fibre, going to the body surface or rather coming from the latter. The transition of the cellular process into the medullated nerve-fibres always takes place at one of the constrictions of **Ranvier** (*re*), hence three medullary segments come together at that point.

Afferent Fibres in the Ganglion.

In many places of our ganglion we can observe another important peculiarity. Frequently we find the nerve-cells surrounded by a more or less dense network of fibrils. This is seen best in those places where the cell-body itself has been stained weakly or not at all. We furthermore notice that a fine, non-medullated nerve-fibre (*mlf*) develops from this network; this fibre does not take on any medullary sheath in its further course. It is a *sympathetic fibre*, the origin of which we will soon be acquainted with (p. II, 336).

PLATE 84

Fig. 183.—Thoracic Ganglion of the Cat

Fig. 184.—From the Thoracic Ganglion of the Cat

Fig. 183.—Thoracic Ganglion of the Cat

25. $\frac{2}{3}$. Vital methylene blue staining.

The process described on p. II, 326 furnishes also material for the study of the sympathetic ganglia. We select any desired ganglion of the sympathetic trunk, preferably the thoracic ganglion, which we can find on either side at the height of the first or second intercostal space, lying upon the longus colli muscle. Paraffin sections are made parallel to the surface, including all the afferent and efferent nerves.

Gross Structure of the Sympathetic Ganglia.

The ganglion is triangular in shape; the cervical sympathetic (*nsy*) enters it at the right, the thoracic sympathetic (*nsy*₂) emerges from it above, three rami communicantes (*rco*) form its communication with the last cervical and the first thoracic nerves and one ramus cardiacus (*rca*) is given off to the heart.

The ganglion is surrounded by a connective tissue fatty capsule, and contains in its interior the sympathetic nerve-cells.

Fig. 184.—From the Thoracic Ganglion of the Cat

300. $\frac{3}{4}$. Vital methylene blue staining.

Elements of the Sympathetic Ganglia.

Examining our specimen with high power, especially where the cells are not too closely packed, we find that the sympathetic cells give a picture very similar to that offered by the cells of the spinal ganglia. They are medium-sized, polymorphous cell-bodies, surrounded by a capsule; they give off numerous, often very strong, dendrites. The dendrites ramify extensively and can be traced for long distances. Each cell sends out one neurite (*n*), which leaves the ganglion as a non-medullated nerve-fibre. The course of the latter varies; it may simply pierce the sympathetic trunk and enter another ganglion, where it communicates with another sympathetic cell, forming an afferent fibre, which terminates at the cell in the form of an end-basket (*af*); or it re-enters the spinal ganglion by way of a ramus communicans, forming the previously recorded (p. II, 333) end-net around one of the ganglia cells, or it becomes a peripheral sympathetic fibre, which may be motor or sensory in character. Our specimen shows that the sympathetic ganglia also contain medullated fibres (*mlrf*). They are probably anterior root-fibres, which gain the ganglion through the ramus communicans and end at the ganglion cells.

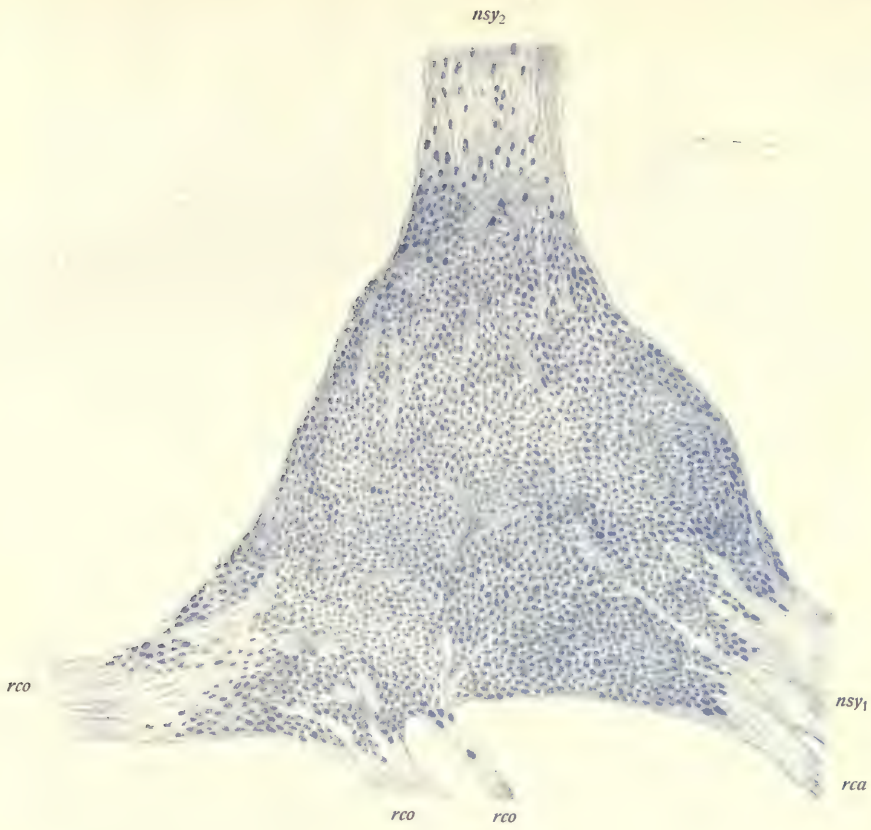


Fig. 183.

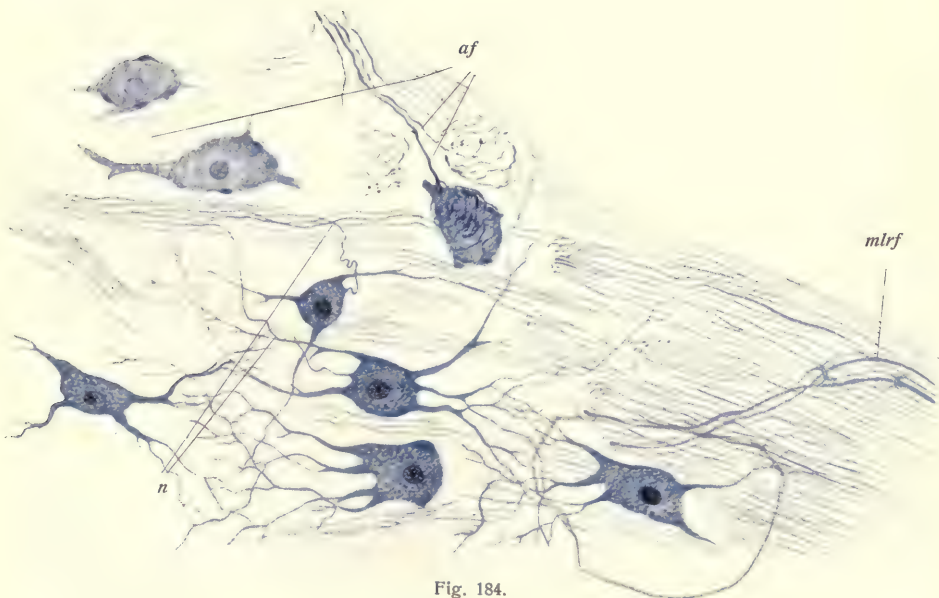


Fig. 184.

PLATE 85

**Fig. 185.—Section through the Anterior Portion of the Human
Eye**

Fig. 186.—Equatorial Sections through the Lens of the Rabbit

8. THE ORGANS OF SENSE

Fig. 185.—Section through the Anterior Portion of the Human Eye

12. Sublimate-platinum chloride. Paraffin section. Carmalum. Picro-indigcarmin.

The preparation of useful specimens of the human eye is quite difficult. The material is hard to obtain, since human eyes cannot, as a rule, be had immediately after death; it is, however, essential to use absolutely fresh material, since the structure of the retina suffers elementary changes soon after death. Other parts of the eye are less easily affected, giving satisfactory specimens twelve to twenty-four hours after death.

The entire globe of the eye is fixed in a mixture of 25 cm³ of a 1% solution of platinum chloride and 25 cm³ of a 5% solution of sublimate, diluted with 50 cm³ of water; the eye is suspended in this liquid for twenty-four hours, washed in running water for an equal length of time, and transferred to 5% formalin for two to three days. The subsequent dehydration must be conducted very carefully and slowly. When the specimen has arrived at the absolute alcohol, it is bisected by an equatorial section into an anterior and a posterior half, both of which are embedded in paraffin. The former is divided into meridional sections, crossing the cornea and the iris. As soon as the lens appears in the section, the cutting becomes difficult, since its substance has turned very hard and brittle. We therefore place alongside of the microtome a tray, containing a mixture of 1 part of soft and 2-3 parts of hard paraffin, which is heated slightly above the melting-point of paraffin by a small burner. The paraffin should have a temperature of 60-70°; thus it must not give off any vapors. A medium-sized camel's hair brush is kept in the tray. Before each section we brush over the surface of the block once, rapidly and evenly, creating a very thin layer of paraffin, covering the entire cutting surface. In this manner we can obtain faultless sections of 10-15 μ thickness, which are pasted on in the usual manner, so that the paraffin coating is on top. The sections are stained in carmalum (p. 55) and counterstained in picro-indigcarmin (p. 67). Sections stained with resorcin fuchsin (p. 63) also furnish very instructive pictures of the distribution of elastic tissue.

Sclera.

The outer covering of the globe, the sclera (*sc*), is very much defined by its bright blue color. The anterior portion here has an average thickness of 0.5 mm, consisting of connective tissue bundles, the majority of which have a meridional course. Between these we find equatorial and oblique bundles.

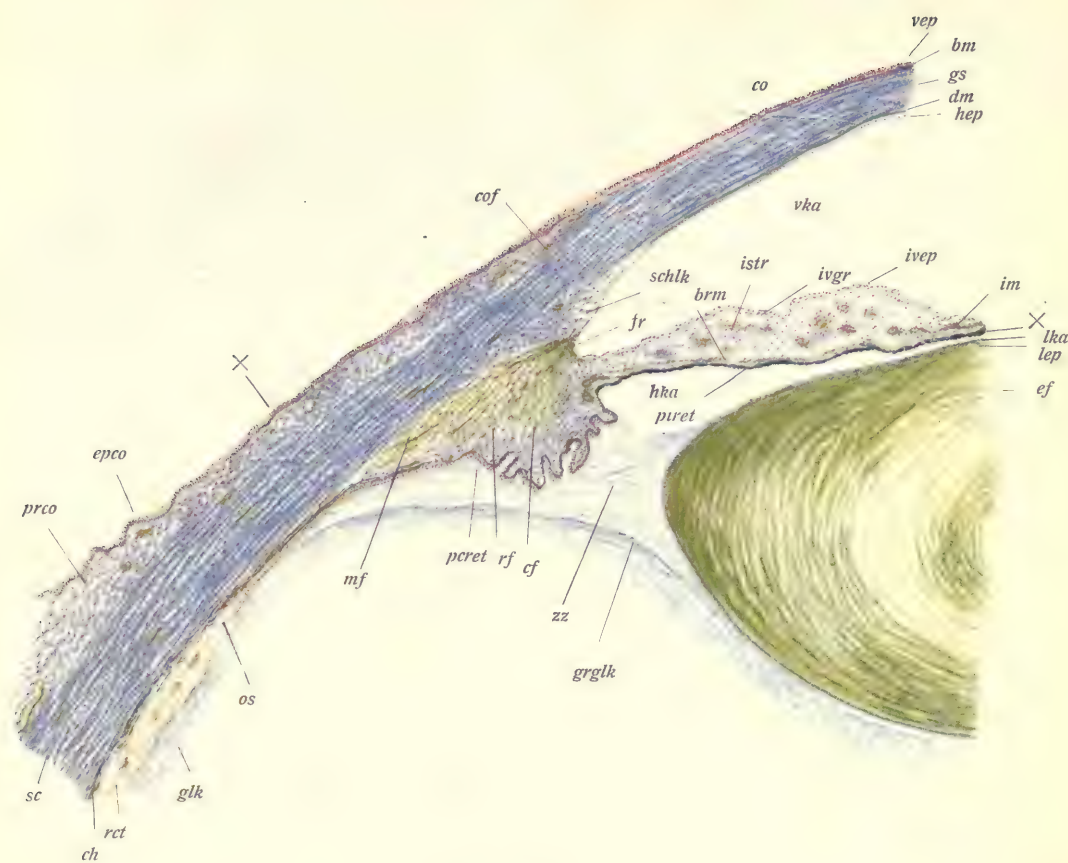


Fig. 185.

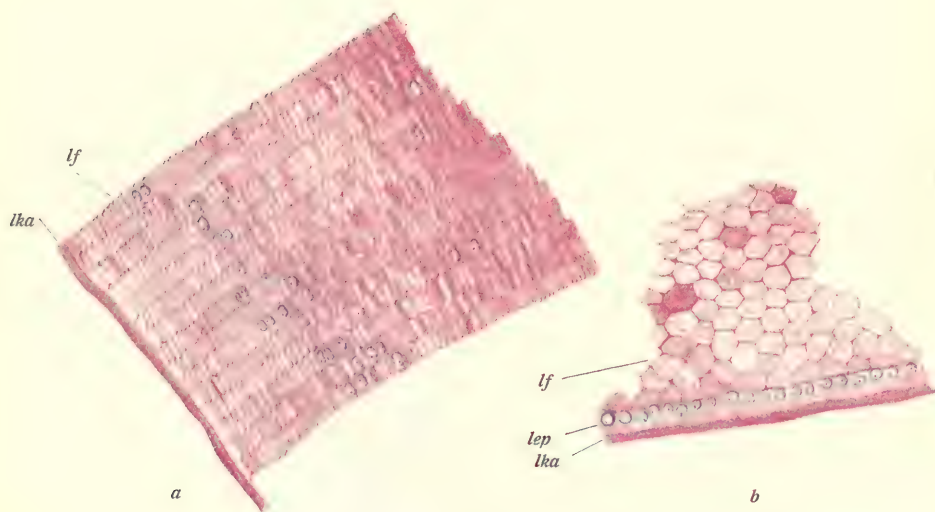


Fig. 186.

They form several layers, leaving narrow spaces between them, which are filled with cells, the *scleral corpuscles*. The resorcin fuchsin method will also demonstrate, especially in the innermost layers, an abundance of elastic fibres. Externally the sclera is surrounded by connective tissue, which is continuous with the propria of the **conjunctiva bulbi** (*prco*). The latter is covered externally by stratified **cylindrical epithelium** (*epco*), which not infrequently contains goblet cells.

Cornea.

The continuation of the sclera anteriorly is formed by the cornea (*co*), particularly by the **basic substance** (*gs*) of the latter. It consists, as the sclera, of connective tissue fibres, which arrange themselves in numerous plates, running parallel to the surface of the cornea. Here, too, we find elastic fibres, but not as numerous as in the sclera. Hence the difference between the sclera and the substantia propria of the cornea is less morphological than physical, the former being opaque, the latter absolutely transparent. Cells, the **cornea corpuscles**, are found in the corneal basic substance; their special peculiarities will be studied in a subsequent specimen. At the point of junction between cornea and sclera, the so-called **scleral** or **corneal groove** (*cof*), we find a slight constriction, the external scleral sulcus.

Externally the substantia propria of the cornea is covered by a thin, variably constructed membrane, which we designate as **Bowman's membrane** (*bm*). or lamina elastica anterior. It is simply a thickened portion of the substantia propria.

This layer is followed by the **anterior epithelium** of the cornea (*vep*), a stratified flat epithelium, which we have met before (p. II, 44).

Posteriorly and toward the anterior chamber, the basic substance is likewise walled off by a homogeneous membrane (*dm*). This **membrane of Descemet**, or lamina elastica posterior, is also a part of the substantia propria, but is much more powerfully developed, always present, and differs microchemically and in its staining properties, as our specimen shows, from the substantia propria. While the latter stains purely blue, the membrane of **Descemet** takes on a green hue. This very resisting membrane offers protection to the eyeball, which should not be underestimated.

Another layer finally surrounds the membrane of **Descemet**, a stratum of simple, low, polygonal cells, the **posterior epithelium** of the cornea, bounding the latter against the anterior chamber.

Chorioid.

The second middle layer, the chorioid coat, contains a large part of the blood-supply of the retina; its structure will be discussed later. Anteriorly it is greatly thickened, becoming continuous with the ciliary body, corpus ciliare.

Ciliary Body.

The thickening is due to an acquisition of **smooth muscle fibres**, which make up the **ciliary muscle**. We distinguish three portions of it. Most externally are fibres which run meridionally, parallel to the internal surface of

the sclera (*mf*); this layer is followed by fibres which radiate in fan-shape from the antero-external portion to the postero-internal region (*rf*), and finally we find, most internally, cross-sections of bundles of fibres (*cf*), which form a muscular ring in the eyeball. The ciliary body is resting on a connective tissue stratum, the so-called **ground plate**. It is differentiated very readily by its blue color from the yellowish-green muscle.

Ciliary Processes.

The ciliary processes arise from it; they are meridionally arranged, comb-shaped folds, the exact form of which is hard to discern in our meridional section. They project into the posterior chamber, consisting mainly of vascular connective tissue.

The Region of the Angle of the Iris.

Still more anteriorly the ciliary body merges into the iris. This region of the angle of the iris, so-called, is worthy of a close examination. Looking with high power at the portion of sclera, in front of the corneoscleral border, we notice that it bulges out internally, forming the **scleral protuberance**. Ascending from the iris, we have connective tissue strands, the **processes of the iris**; they pass the scleral protuberance and at the corneoscleral border they join *Descemet's* membrane. At this point, viz., opposite the scleral protuberance, we find the **canal of Schlemm** (*schlk*), which generally consists of two to three cross-sections of vessels, forming a ring-shaped venous plexus. Externally and posteriorly the processes of the iris form a meshwork of lymph-spaces on the other side of the scleral protuberance, which have been collectively called the **space of Fontana** (*fr*). The canal of *Schlemm* and the space of *Fontana*, viz., the anterior chamber, are in open communication. The canal thus represents the main channel of drainage for the aqueous humor.

Iris.

The iris is a disk-shaped diaphragm, extending from the ciliary body over the anterior surface of the lens. After identifying its shape, we notice that the bulk of the iris is formed by the blue-stained, connective tissue **iris-stroma** (*istr*), which is connected at the root of the iris to the scleral protuberance and the membrane of *Descemet* by the aforementioned processes of the iris. Furthermore, it is in relation with the ground-plate of the ciliary body. Where the ground-plate and the root of the iris merge, we always find numerous cross-sections of vessels, which belong to the **circulus arteriosus iridis major**, derived from the anterior and posterior ciliary vessels. In the remaining stroma of the iris we also find numerous vessels, especially where the ciliary portion of the iris merges into the narrow pupillary part. Here we have the **circulus arteriosus iridis minor**; both plexuses anastomose. High power shows the iris-stroma to consist of loose connective tissue, becoming denser around the blood-vessels. Between the connective tissue bundles are scattered branching pigment cells, which in this case are scantily represented, indicating that the eye of the individual must have been blue or gray.

Smooth muscle fibres (*im*) are also found in the pupillary portion of the iris. They have been cut transversely in part, appearing in bundles, the

sphincter pupillae, partly they form narrow strands, radiating toward the pupillary edge, the **dilatator pupillae**.

Anteriorly the iris-stroma thickens, forming the **anterior border-layer** (*ivgr*), which is separated from the anterior chamber by a simple layer of flat epithelium, the **anterior epithelium** of the iris (*ivep*). The cells of this layer also cover the processes of the iris and are continuous with the posterior epithelium of the cornea.

Retina.

Finally, we arrive at the innermost layer of the globe, the retina, of which our section only shows the anterior portions. We can see but little of the complicated structure of this coat, which will be discussed in detail later; the elements, receiving the impressions of light, the rods and cones, have disappeared. The different layers have merged into one, which mainly consists of supporting tissue, interrupted by blood-vessels and rounded gaps. In the region of the **ora serrata** the retina suddenly decreases materially in thickness, becoming the epithelial covering of the ciliary body.

Pars Ciliaris Retinæ.

This so-called pars ciliaris retinæ covers the ciliary body and its processes completely (*pcret*). Under high power we find that it is made up of two rows of epithelium. Externally we find the ground-plate of the ciliary body covered with a row of cuboid cells, the bodies of which are filled with pigment. They are the direct continuation of the pigment epithelium of the retina. The inner layer consists of slightly higher, non-pigmented cells, which give off blue-stained fibres, which at first form a cuticle over the epithelium, but later course toward the lens, bridging over the recesses between the ciliary processes. In their entirety they form the suspensory ligament of the lens, the **zonula ciliaris** or the **ligament of Zinn**. The cells, mounted on the ciliary processes, often show small vacuoles; they are probably the elements furnishing the aqueous humor for the anterior and posterior chambers. At the root of the iris the pars ciliaris retinæ comes in contact with the posterior surface of the iris, changing them to the pars iridica retinæ (*piret*).

Pars Iridica Retinæ.

This, too, consists of two layers, but here both contain pigment. The cells are so loaded with it that their outlines are hard to distinguish. It is better, therefore, to extract the pigment in the manner described on p. II, 31. We can recognize, then, that the anterior cells give off processes, which are closely approximated, forming a thin layer between the stroma of the iris and the pars iridica retinæ, which has been named **Bruch's membrane**, or posterior border-layer of the iris. In our specimen we see it as a distinct, fine, green line (*brm*). Probably these processes are contractile, which would result in the interesting fact that an ectodermic cell, partly filled with pigment, performs the function of a muscle cell with the non-pigmented part of its body.

Lens.

The lens, the last part of our specimen to be discussed, is not equally well preserved in all its parts. The nucleus of the lens has become more or less

detached from the peripheral portion; the interposed fibrous mass is likewise badly preserved. This cannot be avoided in such total specimens. Externally the lens is bounded by the blue-stained **lenticular capsule** (*lka*). It is thickest in the centre of the anterior surface (anterior lenticular capsule), decreasing in size toward the equator until, in the centre of the posterior surface (posterior lenticular capsule), it is only half the thickness of the anterior central portion.

Below the anterior lenticular capsule we find a simple layer of flat cuboid cells, the **lenticular epithelium** (*lep*). In the region of the equator the cells grow gradually higher and become continuous with the fibres of the lens. The nuclei, receding from the capsule, cause the appearance of the well-known **lenticular whirl**.

Zonule of Zinn.

The fibres of **Zinn's zonula** (*zz*), the origin of which we met before, cross over in a more or less curved course to the anterior and posterior surfaces of the lens and its equator, where they merge into the capsule. Posteriorly the zonule is bounded by the **vitreous** (*glk*). The elements composing the latter, minute gray fibrils, radiate into the vitreous on all sides; at the internal surface of the retina, and the posterior surface of the zonule and the lens, they condense into a sort of membrane, the **border-layer** of the **vitreous body**.

Fig. 186.—Equatorial Sections through the Lens of the Rabbit

(a) Section close to the equator of the lens; (b) section through the anterior half of the lens.

300. $\frac{2}{3}$. Sublimate-platinum chloride. Paraffin section. *Biondi* solution.

One way of preparing specimens for the study of the structure and arrangement of the fibres of the lens is to macerate the lens in alcohol (p. 35), thus dissolving the cement substance connecting the fibres; this method will give us the long, isolated fibres of the lens. Equatorial sections, however, furnish better pictures. Take the fresh eye of a young, not too large mammal, carefully cut into the cornea, near the scleral groove, with the razor, until the aqueous humor exudes. The blunt blade of delicate scissors is introduced into the anterior chamber and the entire cornea is cut off parallel to its periphery. The specimen is fixed in the following solution: 25 cm³ of a 1% solution of platinum chloride, 25 cm³ of a 1% solution of sublimate, and 50 cm³ of water. After two hours the globe can be divided by an equatorial section; the anterior half is replaced and left in the fixing solution until the following day. Further treatment is the same as in the preceding specimen. The sections are made parallel to the corneal edge and stained in *Biondi* solution (p. 67).

Shape and Arrangement of the Fibres of the Lens.

A section, cut closely behind the lenticular equator, will furnish a very regular picture (Fig. 186, a). Externally we have the **lenticular capsule**

(*lka*), internally the **fibres** of the **lens** (*lf*). In cross-section they are seen as regular, narrow hexagons, arranged in rows or, plastically speaking, in leaves, which converge toward the nucleus of the lens. The various leaves are intimately attached among themselves by their serrated edges. Each fibre shows a deep red cortex, while its contents stain in all shades, even remain almost unstained, and again taking such a deep stain at other times that cortex and interior can scarcely be distinguished. The uppermost layer of the lens shows more voluminous fibre-sections, probably representing the thickened ends of the fibres. Numerous fibres show their nuclei.

An entirely different view presents itself in the anterior half of the lens, Fig. 186, *b*. Here the capsule (*lka*) is followed internally by the epithelium (*lep*). Its cells, being cut obliquely, appear much higher than they really are. The oblique section has also affected the fibres, changing the regular, narrow cross-sections into broad, irregular hexagons.

PLATE 86

Fig. 187.—Eye of the Monkey

Fig. 187.—Eye of the Monkey

25. $\frac{3}{4}$. Sublimate-nitric acid. Formalin. Paraffin section. Cresyl violet.

For the examination of the minute structures of the eye, especially the most interesting and important part, the retina, we shall use animal material for reasons stated before. Although fundamentally the retina of all mammalia corresponds to that of man, there is one important point of difference between the human and the animal retina. The human retina is provided in the axis of the eye, viz., at the point whereon the picture is projected, with a depression, the *fovea centralis*. This depression is lacking in the mammal, its place being taken by the so-called *area centralis*, a modified portion of the retina. An exception to this rule is made by the monkey only; his retina assimilates that of man even in this respect. For this reason we have selected the eye of the monkey. By far the best fixative for the retina is a 7.5% solution of nitric acid, to which is added 2.5% of sublimate. After killing the animal by chloroform, we enucleate the globe in the well-known manner, being careful to include a 0.5–1 cm long piece of the optic nerve. The rest of the nerve is fixed in formalin and used for frozen cross-sections. The eyeball is suspended on the stump of the optic nerve in 100 cm³ of the fixing solution for five to six hours, then placed in repeatedly changed 10% formalin for three to five days, and carefully dehydrated by graded alcohols, starting with 10%. It remains twenty-four hours in each of the ten grades of alcohol. In the absolute alcohol we incise the globe. We either divide it by an equatorial section into an anterior and posterior half, or we make two parallel sections, taking off a cap on either side, so that a middle piece, of a centimetre thickness, remains, containing the optic nerve and the lens. The paraffin sections should be so placed that the fovea centralis and the papilla nervi optici are covered in one section; the two former razor sections must therefore be made parallel to this plane. In order to have some topographical points, we may, while the eye is yet in its natural position, mark it before the enucleation with a silver nitrate stick, both externally and internally, at the corneal border, so that a line, connecting the two points, would run from the outer to the inner angle of the eye. These surface markings will even last in the alcohol.

The dehydrated globe is placed in a cork, which has been sufficiently hollowed to receive half of the eyeball, so that the line connecting our two markings coincides with the upper edge of the cork. The section can now easily be placed; of course it is parallel to the surface of the cork; we may either use a sharp razor or scissors and pinch forceps, avoiding all pressure on the globe. The specimen is now placed on a glass plate upon the cut surface, and the second section is made, parallel to the first. During this manipulation it must be kept moist with absolute alcohol, in order to prevent drying. The

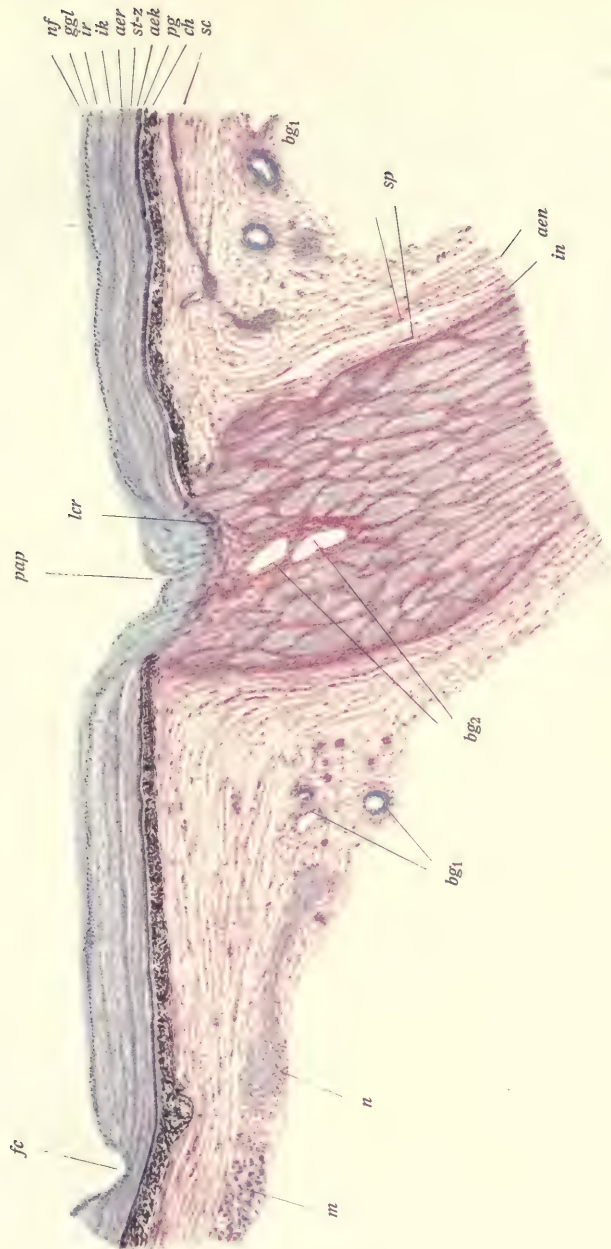


Fig. 187.

middle piece, which most certainly contains the fovea centralis, is replaced in absolute alcohol for twenty-four hours, without removing the vitreous, and embedded in paraffin in the usual manner. The sections are controlled under the microscope or with a strong magnifying glass. Since thus far our final cutting direction has only been approximately defined, the block must be turned during the sectioning until the papilla and the fovea are exactly cut in the same plane. In favorable cases we may thus obtain ten sections of 10 μ thickness, which contain the papilla and the fovea centralis in juxtaposition. They are stained in cresyl violet (p. 62) or *Biondi* solution (p. 67), or after the iron-alum-hæmatoxylin method (p. 57).

Structure of the Optic Nerve.

Our Fig. 187 reproduces the posterior portion of such a section. Turning to the optic nerve, we find it surrounded by two connective tissue sheaths, the **outer neurilemma** (*aen*) and the **inner neurilemma** (*in*). The space left between the two communicates with the subdural space of the cerebrum. A similar space, found within the inner neurilemma, is in communication with the arachnoid spaces of the brain. The entire nerve is divided into numerous longitudinal compartments by septa, which are given off from the inner neurilemma. The bundles of medullated fibres contained therein are cut partly obliquely, partly longitudinally; they surround a central, connective tissue cord, which harbors the central artery and vein of the retina (*bg₂*). They have also been cut obliquely. At its entrance into the globe the optic nerve seems constricted, because here its fibres lose their medullary sheath. The loss of the medullary sheath is also expressed in a change in color in our specimen, really more striking than is reproduced in our drawing; the medullated portion of the fibre appears bluish-red, the non-medullated part purely blue.

Entrance of the Optic Nerve Into the Globe.

At the point of constriction the fibres must pass through the sieve of the **lamina cribrosa** (*lcr*), which, coming from the sclera, pierces into the nerve with its numerous connective tissue fascicles, dividing the fairly thick bundles into many smaller ones. The latter spread over the inner surface of the retina at the height of the posterior retinal surface, forming the funnel-shaped **papilla nervi optici**. Other sections will show us the distribution of the central vessels from the papilla over the retinal surface.

Layers of the Retina.

The retina, which is wanting in the region of the papilla, is closely approximated to the strongly pigmented chorioid coat. Low power already reveals the well-known layers, which stratify the organ, nucleated layers alternating with non-nucleated. From within outward they are: nerve-fibre-layer (*nf*), ganglia cell-layer (*ggl*), inner reticulated layer (*ir*), inner granular layer (*ik*), outer reticulated layer (*aer*), outer granular layer (*ack*), layer of rods and cones (*st-z*), and pigmented epithelium (*pg*). Of the outer reticulated layer we may distinguish a separate, external, light-blue band, which is often spoken of as *Henle's* fibrous layer. In addition to those men-

tioned we have two border membranes, a *membrana limitans interna*, which separates the retina against the vitreus, and a *membrana limitans externa*, between the outer granular and the layer of rods and cones.

Fovea Centralis.

About 4 mm from the papilla in a temporal direction we come to the fovea centralis, a funnel-shaped depression, on the floor of which we find, of all the retinal layers, only the pigmented epithelium, the rods and cones, the outer granular and the outer reticulated layers.

The Chorioid.

Under high power we can divide the chorioid into an outer, strongly pigmented ***chorioidea propria*** and an inner ***choriocapillaris***, which is almost free from pigment; the former contains the coarser vascular branches, the latter the capillaries. The latter is bounded internally by the ***lamina basalis***, externally by the ***lamina suprachorioidea***, which forms part of the sclera.

The connective tissue of the orbit, which is separated from the sclera by a space, not always visible, is partly shown in our specimen; it is rich in fat, contains the muscles (*m*), ciliary nerves (*n*), and blood-vessels (*bg*₁).

PLATE 87

Fig. 188.—Retina of Monkey

Fig. 188.—Retina of Monkey

550. Sublimate-nitric acid. Paraffin section. *Biondi* solution.

For a close examination of the retina we stain a 5–10 μ thick paraffin section of the previous specimen in *Biondi* solution (p. 67). We search for a place near the fovea centralis and study it with homogeneous immersion.

Only a small portion of the chorioid is included in our field. We see the *chorioidea propria* with its numerous large blood-vessels and its pigmented cells. Internally it is followed by the **choriocapillaris**, its homogeneous, pink basic substance containing many capillaries. Internally the chorioid coat is bounded by a thin, bright red *lamina basalis*.

Pigment Epithelium.

The outer retinal layer (*pep*) shows the well-known cuboid pigment cells (p. 31). The pigment is scanty in our specimen. It extends in fine threads between the rods and cones.

Neuroepithelial Layer.

The neuroepithelial layer (*st-z*) follows; it presents a mass of **rods** and numerous **cones**; two cones are always separated by two or only one rod. This shows that our specimen has been taken from the central portion of the retina; the nearer we come to the periphery, the less numerous will be the cones. Shape and structure of the rods and cones are well demonstrated in our section. We are able to distinguish between an **inner** and an **outer segment**. The inner segment of the rod (*igst*) is about equally long, but considerably thinner than that of the cone (*iglz*). Both stain intensively red, the outer portion of the inner segment being the deepest red. Enclosed in the inner segment of the rod as well as of the cone we have an oval body, the **ellipsoid** (*elz*). The latter often shows a longitudinally striated, fibrous structure. The outer segment of the rod (*aglst*) is a long, thin thread, extending to the pigment epithelium. The outer segment of the cone (*aglz*), on the contrary, is a short, conical peg, mounted on the inner segment, staining weaker than the outer segment; the latter is also true of the outer segment of the rod.

Outer Granular Layer.

The rods and the inner segments of the cones are mounted by their base on the **limitans externa** (*le*). It can be noticed with difficulty in our specimen that they do not end there, but continue into the granular layer. This can be especially said of the cone. It swells on the other side of the limitans, forming a cell-body, which incloses a nucleus, situated closely underneath the limitans. Beyond the nucleus the cell narrows down to a strong

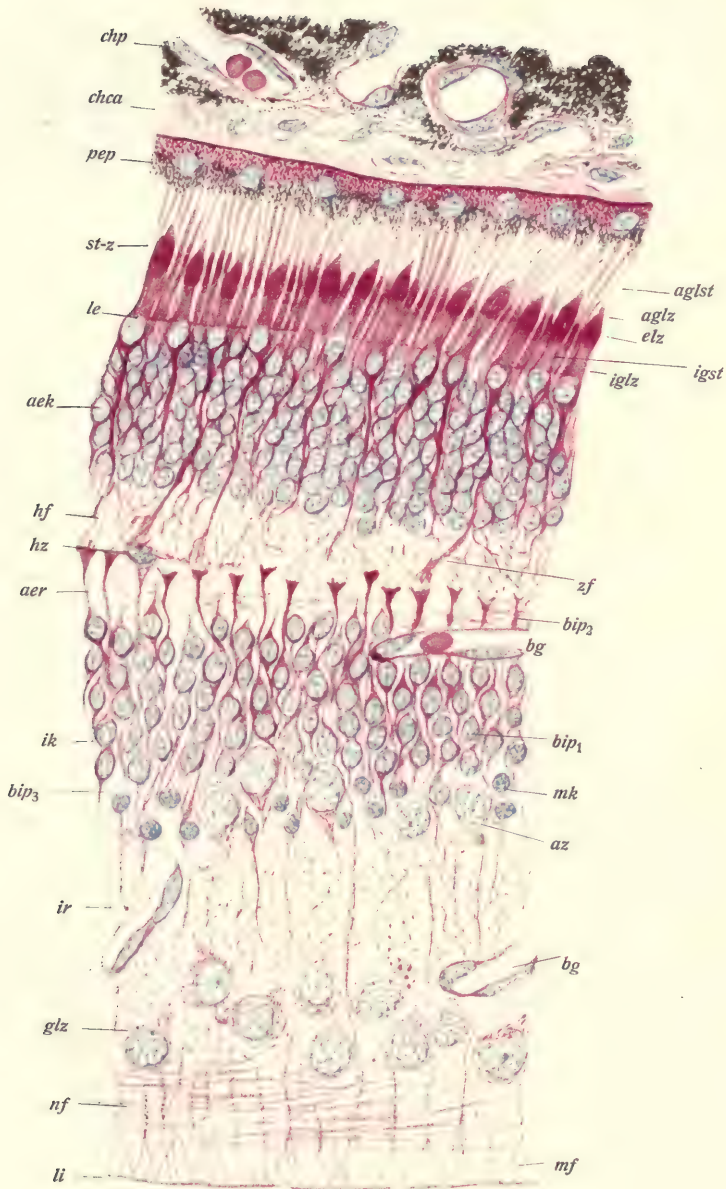


Fig. 188.

process, which can be traced through the outer granular and *Henle's* fibrous layers into the outer reticulated layer, where it ends in a footlike thickening (zf). The entire formation is called a **cone-optic cell**, consisting of an outer segment, an inner segment, including an ellipsoid, the cone-grain, cone-fibre and cone-foot. The rod is likewise only a part of a **rod-optic cell**, but we hardly succeed in our specimen to distinguish all its different parts. Beyond the limitans we might possibly recognize the continuation of the inner segment into a thin fibre and the relation of the latter with the rod-cone, but after that our specimen fails to give further information. The nuclei of the rod-optic cells form the main bulk of the nuclei, constituting the outer granular layer, being found in all regions of this stratum, while the nuclei of the rod-cells are only found closely under the limitans. The latter are smaller than the former.

Henle's Fibrous Layer.

The fibrous layer of *Henle* is mainly formed by the oblique or vertical cone-fibres, but also contains peculiar cells, one being shown in our section (hz). These cells have processes, which mainly spread parallel to the surface of the retina, connecting distant parts of the latter; they are called **horizontal cells**.

Outer Reticulated Layer.

This stratum (*aer*), aside from the horizontal cells, contains the peripheral processes of the bipolars, which will be considered presently. This layer, in the peripheral portions of the retina, where few cones are present, cannot be differentiated from the preceding, the two merging into one

Inner Granular Layer.

The inner granular layer presents mainly the so-called **bipolars of the rods and cones**. The cell-body (*bip₁*) surrounds a round or oval nucleus; it sends a fairly strong process (*bip₂*) peripherally into the outer granular layer, which ends in the outer reticulated or in *Henle's* fibrous layer in a footpiece. Centrally the cell-body gives off a finer process (*bip₃*), which, in our specimen, becomes lost in the inner reticulated layer. Furthermore we find, especially in the deeper regions of the inner granular layer, cells containing large nuclei; they are the **amakrine-cells** (*az*); about their form nothing definite can be learned from our specimen. Blood-vessels are found in the inner granular layer, as well as in the deeper layers. They are branches of the central artery and vein of the retina, which we met when discussing the papilla nervi optici; piercing through the latter, they reach the inner surface of the retina. In other sections we will meet their capillaries in the outer granular layer. They never trespass on the limitans externa, leaving the neuroepithelium always avascular.

Inner Reticulated Layer.

The subsequent inner reticular layer forms a chaos of fibres, which resist any segregation. Only one kind of fibres can be followed with some sort of certainty. They are thicker or thinner fibres, crossing the layer transversely and enlarging within the inner granular layer to form a nucleated

cell-body. These *Mueller's* fibres (*mf*) are the supporting elements of the retina. They arise by several feet, as our section illustrates very nicely, from the limitans interna (*li*), cross every retinal layer up to the neuroepithelium, ending in the limitans externa. Their entire course can be traced in sections taken from the peripheral regions of the retina. The two limitantes are simply the outer and inner endings of *Mueller's* fibres, intimately united in planes. *Mueller's* fibres thus serve to support the organ and to isolate its elements.

Layer of the Ganglia Cells.

In the ganglia layer, following externally, we find many large **ganglia cells**. Their body is rounded or edged, and always contains the basophilic granules, which we have met before (page II, 121). The nucleus is large, round and shows distinctly basophilic chromatin. The nearer the section to the macula, the more numerous will be these cells, until finally they become stratified in several rows. Toward the periphery they become scanty, lying singly at greater distances and finally disappear altogether.

Nerve-Fibre Layer.

This last layer of the retina is fairly broad in our specimen; it grows narrower as we recede from the papilla nervi optici. It consists of non-medullated nerve-fibres, as we learned during our previous study of this papilla.

PLATE 88

Fig. 189.—Retina of the Guinea-Pig

Fig. 190.—Ganglia Cells from the Retina of the Guinea-Pig

Fig. 189.—Retina of the Guinea-Pig

600. Vital methylene blue staining. Chop method.

While the preceding specimen has given us quite an explicit demonstration of the wonderfully complicated structure of the retina, a few points remain which deserve further elucidation. To complete our study, we will therefore employ the vital methylene blue process, which, when used on proper animals in the correct manner, probably furnishes the best specimens for demonstrating the structure of the retina. Cat and rabbit seldom yield useful specimens, the guinea-pig, on the other hand, almost always gives good results. It is difficult to say why this should be so; probably it is due to the manner of blood-supply of the retina. The process is conducted in the manner described on p. 60, observing the fact that too much dye cannot be possibly introduced into the animal body. The conjunctiva must appear dark blue. After the injection the animal is again left undisturbed for half an hour. The globes are then enucleated and bisected into an anterior and posterior half by an equatorial section, which is started by the razor about 2-3 mm behind the corneoscleral border and finished with the scissors. The posterior hemisphere is grasped with forceps at the stump of the optic nerve, and the latter cut off with the scissors so closely to the sclera that a part of that structure is included. After transferring the specimen in a large vessel, containing *Ringer's* fluid, at body temperature, we find that light shaking with forceps will soon cause the retina and the adhering vitreous to become detached from the globe. They are taken out with a large slide or a flat watch-glass in such a manner that the retina is below. With curved scissors we trim off the vitreous and then place the specimen in a moist chamber. At first it appears diffusely blue, the staining of the nerve-cells and fibres beginning only after fifteen to thirty minutes and reaching its optimum within two to three hours, after which it declines. When the ganglia cells and their branches appear deep blue, and rods and cones can be recognized in the folds, the specimen, together with its support, is placed in a vessel of cold ammonium-molybdate solution (p. 62), where it remains overnight. The following morning we transfer for one to two hours to repeatedly changed water. Small pieces of the organ may now be excised, chopped on the slide with the razor (p. 36), and mounted in levulose, or we may take out the retina with a large slide, spread it well and extract the water with tissue paper. Harden the specimen by dropping upon it 50%, 70% and 95% alcohol successively, then place in absolute alcohol for complete dehydration and later in xylol. Chopped specimens, treated in this manner and mounted in Canada balsam, are much more durable and also more transparent than levulose specimens. Often, however, we must contend with considerable shrinkage.

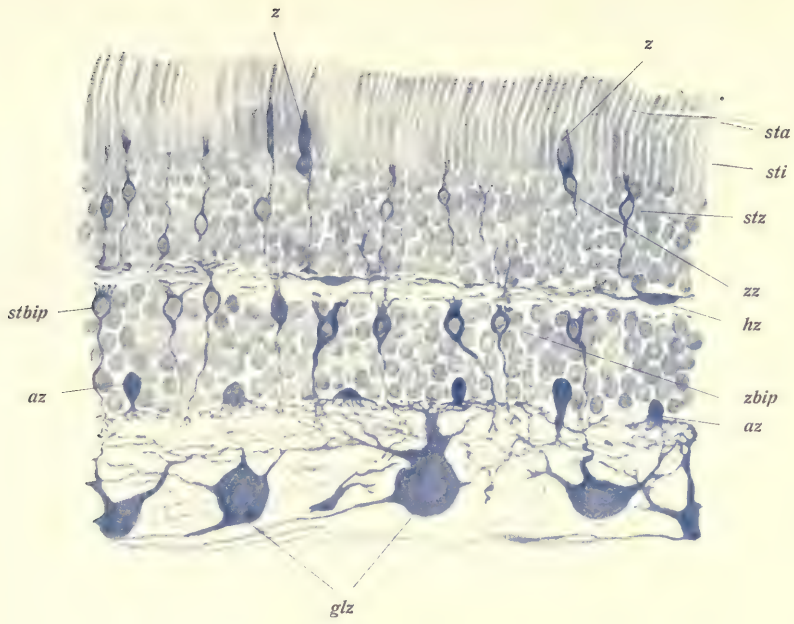


Fig. 189.

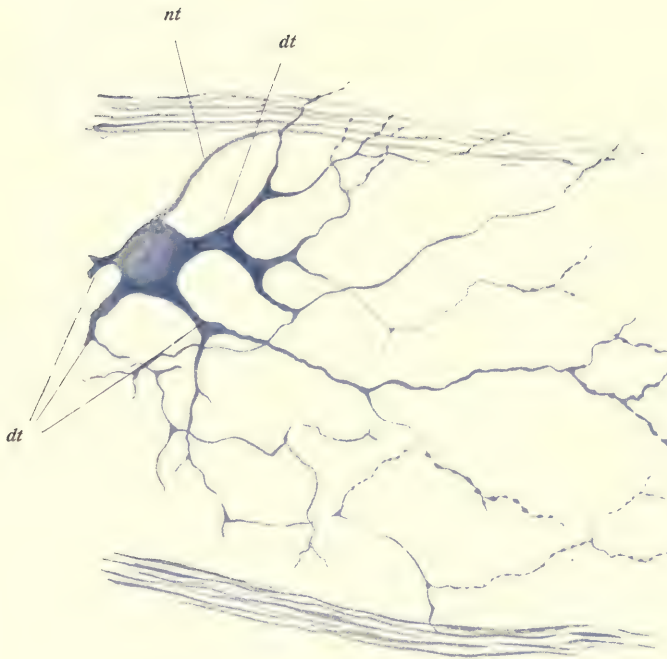


Fig. 190.

*Details of the
Structure of the Retina.*

Surface views as well as transverse are present in a well-chopped specimen. Our picture shows one of the latter. We are surprised by the minimal thickness of the organ. Outer and inner granular layers are separated by only a very thin fibrous and reticular layer. Upon the background of light blue nuclei we find a few cells, stained in intense blue color, which can be traced in all their ramifications. A new structural peculiarity presents itself on the **outer segments of the rods** (*sta*). They appear transversely striated, as though composed of superimposed plates. What we really have is a postmortal maceration, induced by the long interval between the death of the animal and the fixation of the material. Rods and cones are seldom stained throughout. The specimen shows, better than our drawing reproduces, how small fibrils rise from the bodies of the rods, respectively **cone-optical cells** (*stz*, *zz*), which, densely surrounding the **inner segment** (*sti*, *z*), advance into the outer segment. The rod-fibres and cone-fibres are always very distinctly seen. The former end in the inner reticulated layer in small, button-like swellings, the latter in the previously mentioned foot-ending.

Horizontal cells (*hz*) are represented in large amounts. Their processes can be traced far, surrounding in their course the button-shaped ends of the rod-fibres and the feet of the cones.

The rod and cone bipolars, found in the inner granular layer, do not differ much among themselves, but are slightly different in shape from those of the monkey. The peripheral process is considerably shorter, often double; from its end arise numerous fine filaments, which appear to contain the end-granules of the rod-fibres or one foot of a cone-fibre. The central process, which is always thinner in the rod bipolars than in the cone-bipolars, can be traced into the inner reticulated layer, where it terminates in short, claw-shaped branches.

Very important information is given by our specimen regarding the amakrine cells and the ganglia cells. The **amakrine cells** (*az*) are especially found in the deeper regions of the inner granular layer; the cell-body is rounded, pyriform or hood-shaped. It either gives off one thick process, which breaks up in the inner reticulated layer into numerous, horizontally spread branches, or it gives off several processes directly from the body of the cell, which penetrate the inner reticulated layer in a different manner. Since the horizontal spreading of the first-mentioned amakrines takes place at different heights of the inner reticulated layer, these cells are also called the stratifying in contradistinction to the other diffuse amakrines. While in all other retinal cells, corresponding to most all true nerve-cells, we can divide the processes into dendrite- and neurite-formed, or such that have a centripetal and a centrifugal course, such classification cannot be maintained in the amakrines, since they possess but one sort of process.

The **ganglia cells** (*glz*) of the retina are shown here in their true form. They are huge, rounded, oval or irregular cell-bodies, sending out several strong dendrites, the ramifications of which largely constitute the fibrous chaos of the inner reticulated layer. Here the dendrites are in intimate relation with the processes of the amakrines and furthermore with the central

processes of the rod and cone bipolars. Each ganglia cell sends a neurite to the inner surface of the retina, into the nerve-fibre layer, which then constitutes a fibre of that layer; the fibres of the optic nerve are thus simply the nerve-processes of the ganglia cells of the retina.

Fig. 190.—Ganglia Cells from the Retina of the Guinea-Pig

600. Vital methylene blue process.

The numerous surface views found in our specimen also offer interesting pictures of the retina. Our picture shows a ganglia cell and its process. We are now able to appreciate how large an area is covered by such a cell, how it collects stimuli in its body from remote portions of the retina, which are conveyed to its dendrites (*dt*) by the central processes of the rod-bipolars. The very minute branches of the dendrites generally appear beaded, as if studded with varicosities. These formations are artifacts, due to disintegration of fine nerve-fibrils.

The fibres of the nerve-fibre layer are arranged in thinner or thicker bundles, into which the ganglia cells send their neurites (*nt*).

PLATE 89

Fig. 191.—Cornea of the Sheep

Fig. 192.—Cornea of the Cat

Fig. 191.—Cornea of the Sheep

300. $\frac{3}{4}$. Gold chloride-citric acid. Formalin. Frozen section.

Corneal Corpuscles.

Two additional specimens will acquaint us with some details regarding the structure of the cornea. The cornea of a large animal or of man is treated with the gold chloride-citric acid method, as described on p. 72. The cornea of the sheep, for instance, must remain at least fifteen minutes in a large quantity of citric acid, and not less than an hour in the gold solution. Under favorable light conditions the reduction should be completed in twenty-four hours. We transfer to 5% formalin for twenty-four to forty-eight hours, incise the organ from the periphery to the centre, and freeze it on the freezing cylinder of the microtome. The sections, which cover the entire surface of the cornea, are mounted in levulose.

Previously we found numerous nuclei in the corneal substantia propria without receiving any information as to the shape of their cells, the corneal corpuscles. Our specimen shows them to be stellate cells. Numerous processes leave the flat cell-body, which anastomose with their neighbors, thus forming a rich cellular net, covering the entire substantia propria. This cellular net is suspended in a system of canals, formed by the connective tissue of the substantia propria, which is of vast importance for the nutrition of the avascular cornea.

The nerves of the cornea are also demonstrated quite well by the gilding process, but will receive special consideration in a subsequent specimen.

Fig. 192.—Cornea of the Cat

150. $\frac{3}{4}$. Vital methylene blue staining.

Nerves of the Cornea.

In no other portion of the body can the nerves be demonstrated so well by the vital methylene blue process as in the cornea, the latter offering the best object in all such experiments, as have been described or are yet to be considered. To avoid a drying of the outer corneal layers, we recommend moistening the organ from time to time with *Ringer's* fluid; a still better plan is to close the eyelids with artery clips during the process (p. 76). After enucleating the globe, the anterior portion of it is cut off; lens, ciliary body and iris are removed with tissue forceps. After the cornea has been flattened by a radial section it is placed on a slide, moistened with *Ringer's* fluid, and transferred to the moist chamber for bluing. The latter takes place in a

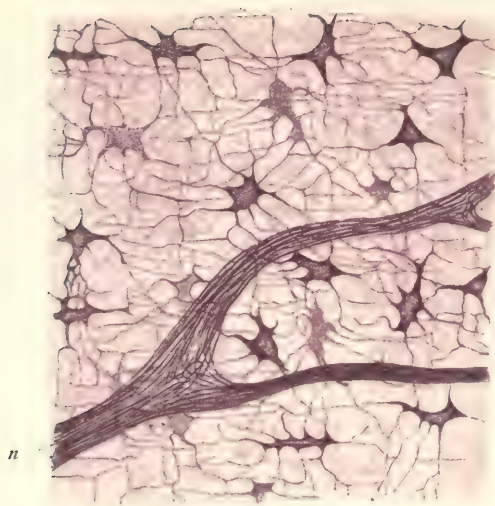


Fig. 191.



Fig. 192.

short while. Fixation and dehydration, as usual (p. 62). The best pictures are obtained from the cornea of small animals, up to and including the cat. The entire specimen is brought under cover with Canada balsam; a lead-ball may be placed on the cover-glass for a few days, to flatten the cornea.

A large amount of nerve-trunks are seen, radiating from the sclera into the cornea, one of which (*n*) is reproduced in our picture. Between the nerve-trunks we also see, coming from the pigmented **sclera** (*p*), blood-capillaries, entering the corneal basic substance, where they terminate in loops (*cschl*). The nerve-trunk at once breaks up into a coarsely meshed **basic plexus** situated in the deep corneal layers (*grpl*). From it the fibres rise toward the epithelium, pierce *Bowman's* membrane, and form a secondary, **subepithelial plexus** (*seapl*), whence the fibrils enter the epithelium (*ep*), ramify between the cells of the latter and terminate freely. Our picture shows all these formations, projected in one plane. The fine network represents the blue-stained cement substance of the deepest layer of the epithelium. The termination of the fibres of the subepithelial plexus between the cells of the anterior corneal epithelium can be studied more conveniently on cross-sections, which are made of the cornea of the other side, previously embedded in paraffin.

PLATE 90

Fig. 193.—Upper Eyelid of Child

Fig. 193.—Upper Eyelid of Child

15. Formalin. Frozen section. Carmalum. Sudan. Picroindigcarmin.

The best objects for the study of the eyelid are obtained from the lid of the child, fixed in 10% formalin. Frozen sections are stained in carmalum (p. 55) for fifteen minutes, then counterstained equally long in an alcoholic solution of sudan (p. 66), and ten minutes in picroindigcarmin (p. 67). After rinsing in water, we mount in levulose.

Structure of the Eyelid.

Our section shows a **cutaneous portion** and a **conjunctival portion**. Between these two we find striated muscle and adipose connective tissue. The cutaneous portion shows all the peculiarities, with which we will be acquainted later, when discussing the body integument. It is covered by a stratified horny epithelium, the **epidermis** (*epid*). The latter also encloses the free edge of the lid, merging on the other side of it, at the inner surface of the lid (X) into the epithelium of the conjunctiva. The epidermis is followed internally by the **corium** (*co*), which is flatly approximated to the former, without the formation of papillæ. This layer as well as the succeeding **subcutis** are composed of connective tissue. The cutaneous part of the lid contains numerous small hairs and sebaceous glands. We also find numerous blood-vessels (*bg*), with their deep yellow contents and bright red fat-lobules (*f*). At the outer edge of the lid **cilia** (*ci*) project from the cutaneous portion, strong hairs, arranged in two or three rows. Their follicles extend into the muscular layer and are accompanied by sebaceous and sudoriferous glands, the so-called **glands of Moll** (*modr*), which differ from the ordinary sweat-glands of the skin by their greater tortuosity and wider lumina; they open into the outer end of the ciliary follicles.

The mucous membrane of the lid, the conjunctiva palpebræ, lines the inner surface of the lid, merging into the conjunctiva bulbi at the fornix. It is covered by a simple layer of **cylindrical epithelium** (*epco*), which merges into the epidermis of the lid at the place marked (X), not at the inner edge of the lid. The conjunctival epithelium always contains numerous goblet cells. It is mounted on a connective tissue *propria*, which is better developed in the fornix of the lid, i.e., the upper third of our specimen; in the lower two-thirds it soon merges into a powerful, thick, connective tissue plate, the **cartilage of the lid** or the **tarsus** (*t*). This formation has nothing in common with cartilage, consisting merely of thick, tightly cemented connective tissue fibres. Hence it stains deep blue. Within the tarsus we notice an elongated **Meibomian gland** (*mbdr*), studded with numerous lateral alveoli. This is merely an immensely developed sebaceous gland; its

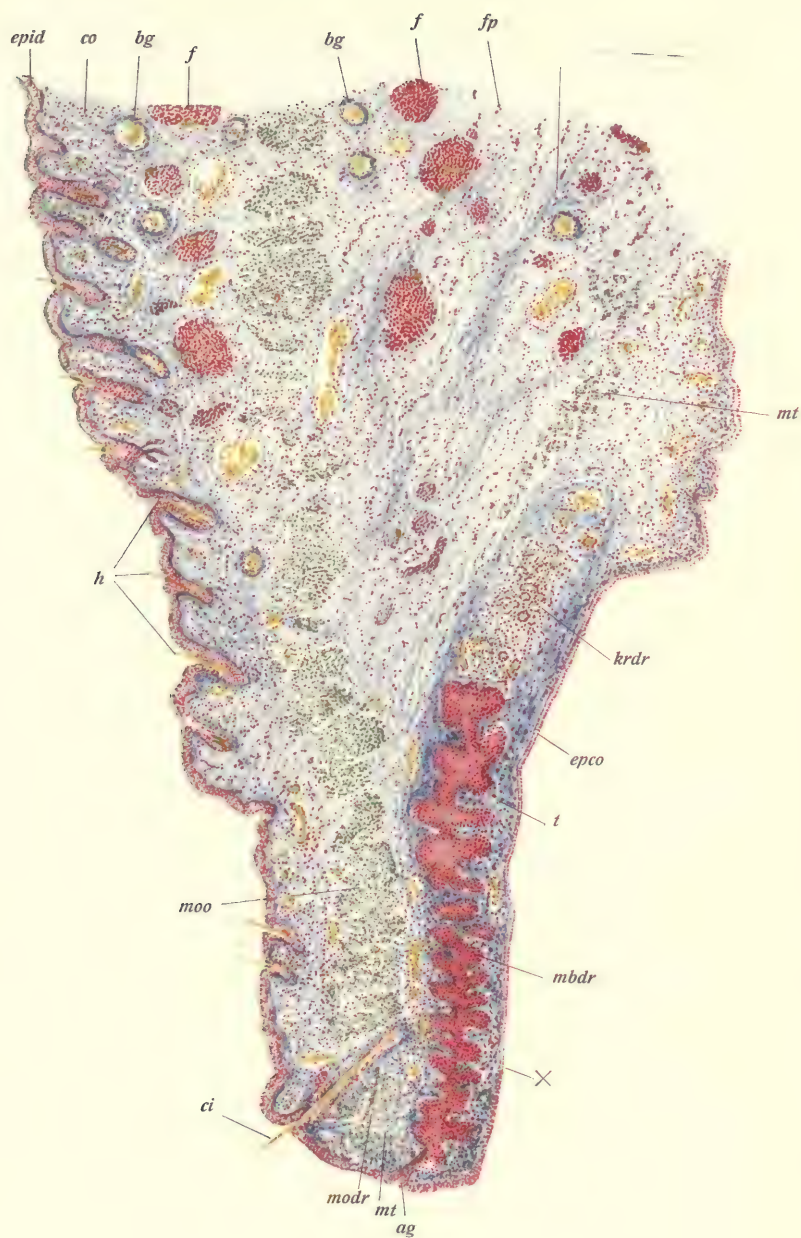


Fig. 193.

fatty contents are stained intensely red with sudan; they represent what is called eyebutter. It opens (*ag*) externally to the inner edge of the lid. Above the *Meibomian* glands we find masses of tubular glands with light cells, the ducts of which open at the surface of the conjunctiva. They have been called **accessory tear glands** (*atr*), or **Krause's glands**.

Between the cutaneous and the conjunctival portion we find an interposed striated muscle, the **orbicularis oculi** (*moo*). The fibres are cut transversely, uniting to form larger or smaller bundles, which are arranged in a single row, crossing the entire width of the lid. The lowest part of the muscle, situated between the cilia and the edge of the lid, is termed the ciliary muscle of *Riolan*. The muscle is separated from the palpebral conjunctiva by a fatty, partly thickened connective tissue, the *palpebral fascia* (*fp*). It also contains obliquely cut, smooth muscle fibres, the *tarsal muscle* (*mt*). The fascia gives attachment to the terminal tendon fibres of the levator palpebræ superioris.

PLATE 91

Fig. 194.—Auditory Organ of a Young Cat

Fig. 195.—Ductus Cochlearis of a Young Cat

Fig. 194.—Auditory Organ of a Young Cat

15. $\frac{3}{4}$. Chromic-osmic-acetic acid. Paraffin section. Iron-alum-hæmatoxylin.

The technical preparation of the auditory organ is probably even more difficult than that of the optic organ. The membranous labyrinth, which contains the most important part, the sensory receiving station, is, in the adult human being, situated deeply in the petrous portion of the temporal bone, thus being hardly accessible to the fixing solutions. The younger the individual, the better are our chances for technical work. The essential parts of the membranous labyrinth, as regards structure and arrangement, show very similar conditions in the mammal to those in man. For these reasons we will select the easily secured and comparatively easily prepared auditory organs of kittens, during the first few days after birth. The technique of taking out and opening the organ has been previously discussed (p. II, 144). The specimen is suspended for three to five days in about 50 cm.³ of a chromic-osmic-acetic acid solution (p. 30), the solution being renewed on the second and possibly also on the third day. After washing in running water for twenty-four hours, we dehydrate cautiously in graded alcohols and embed in paraffin. Previously to embedding we make sure of the direction of section, since it is of importance, to cut the cochlea in the axis of the modiolus; only those sections which go through this plane will furnish useful specimens for the beginner. After pasting the paraffin sections on, we stain them after the iron-alum-hæmatoxylin method (p. 57). The various sections, of course, give different pictures. For our description we select a section, going straight through the centre of the modiolus.

Gross Structure of the Cochlea.

The greatest part of our section is occupied by the cochlea, which is surrounded by a very thin, bony capsule (*k*). A short spacious canal, the meatus acusticus internus (*pai*) enters the latter. It harbors the **auditory nerve** with its two divisions, the **cochlear nerve** (*nc*), shown in longitudinal section, and the **vestibular nerve**, presented in cross-section. The former ascends in the hollow axis of the cochlea, the **modiolus**. In this case it is quite an undeveloped bony canal, giving off a bony leaf, winding spirally within the cochlea, the **bony septum** (*zw*), which communicates with the cochlear capsule. Thus we have the formation of the bony cochlear canal, which, as we know, emerges from the bony vestibulum and forms two and one-half turns within the cochlea.

Besides this bony septum, we see a second bony shelf projecting from the modiolus outward, the **lamina spiralis ossea**. It does not reach the

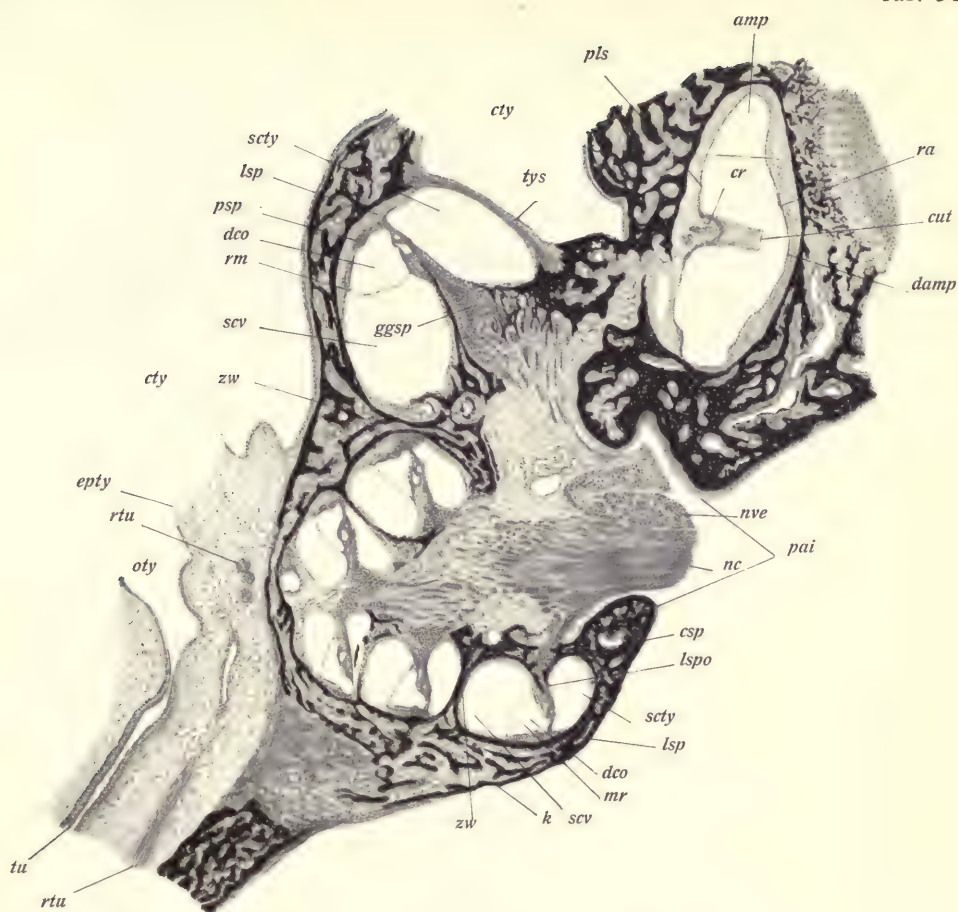


Fig. 194.

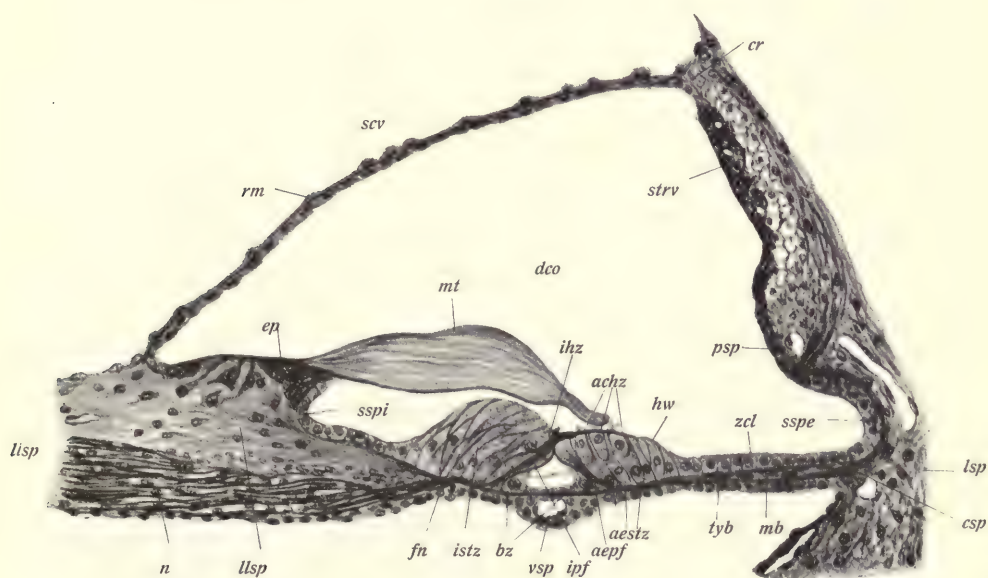


Fig. 195.

outer wall, projecting only part of the way into the bony cochlear canal. In our specimen it is but weakly developed, reaching its best development in the basal turn (*lspo*). Here we can recognize very plainly that it consists of two leaves. There is a small canal left between the two leaves, which opens posteriorly into a somewhat wider canal situated in the wall of the modiolus, ascending spirally within the same. The latter is the **spiral canal** (*csp*). It contains the cellular mass of the **ganglion spirale** (*ggsp*), which internally is in connection with the cochlear nerve and externally sends its fibres into the cochlear canal.

The lamina spiralis ossea is covered by a pad of connective tissue, the **limbus laminae spiralis**; tapering, it reaches the outer wall and merges into a triangular mass of connective tissue, covering the interior surface of the outer wall of the cochlea, the **ligamentum spirale** (*lsp*). An additional, much thinner membrane, *Reissner's* membrane (*mr*), stretching from the limbus laminae spiralis obliquely over to the other end of the ligamentum spirale, helps to divide the interior of the bony cochlear canal into three adjacent canal-like spaces. The **scala tympani** (*scety*) starts blindly at the **membrana tympani secundaria** (*tys*), covering the round window; it ascends spirally in the cochlear canal, merging at the summit of the cochlea into the **scala vestibuli** (*scv*), which descends in a similar manner and opens into a lymph-space, situated between the membranous labyrinth and the lateral wall of the bony vestibulum (within the latter), the **cisterna perilymphatica**. Between the two scalæ a third canal appears, the membranous cochlear canal, **ductus cochlearis** (*dco*). It starts blindly in the recessus cochlearis of the vestibulum, runs between the two scalæ to the apex of the cochlea, where it ends blindly. As we know, it communicates by a narrow canal, the *canalis reuniens*, with the sacculus.

The ductus cochlearis is the most important part of the membranous labyrinth. It contains the auditory end-apparatus, **Corti's organ**. The latter appears as a mass of cells in each turn of the cochlea, being implanted upon the *membrana basilaris*, i.e., the tympanic wall of the cochlear duct; in its entirety it thus forms a spiral band. The blind end of the vestibule and the blind end of the cupula cochleæ lack these nerve-endings.

Eustachian Tube and Tympanic Cavity.

At the left of the cochlear apex a narrow tube opens, lined by a double row of ciliated, cylindrical epithelium, the **tuba auditiva** (*tu*). Blood-vessels and nerves (*ramus tubæ* (*rtu*)) appear in its wall. At the **ostium tympanicum** (*oty*) the high tubal epithelium changes into the low, cuboid epithelium (*epty*) of the **tympanic cavity** (*cty*).

Bony and Membranous Ampulla.

Of the remaining parts of the membranous auditory organ our section shows an ampulla (*amp*). In the young specimen it fills the bony ampulla almost entirely. Later on this condition changes, a cleft remaining between the roof of the membranous ampulla (*damp*) and the wall of the bony ampulla, a perilymphatic space, which communicates with the corresponding

spaces in the semicircular canals and with the cisterna perilymphatica of the vestibule. The beginning of this cleft can be seen here at one place, the ampulla wall, a thin, connective tissue membrane, becoming detached from the periosteum of the bony ampulla; only at the floor of the ampulla they always remain in contact; here the ampullar wall forms a protuberance, appearing like a peg, but really being more of the nature of a moulding, the **crista acustica** (*cr*); the crista is based on a low pedestal, the **planum semilunare** (*pls*). The nerve of the ampulla, a branch of the vestibular, enters the ampulla at this point, ending in the epithelium, covering the crista acustica. This epithelium is, as we may observe, under high power, a sensory epithelium. It consists of cylindrical, **supporting or stay cells**, which extend across the entire thickness of the epithelium, and of bottle-shaped **hair-cells**, which extend from the surface to about the centre of the layer, sending out fine, long hairs from their free ends, which are in close approximation, forming the peculiar, hood-shaped **cupula terminalis** (*cut*) of the crista. At the sides of the crista the sensory epithelium gradually merges into the simple cylindrical epithelium of the planum semilunare, which changes to the simple flat epithelium, covering the entire remaining portion of the ampulla as well as the semicircular canals, the sacculus and the utriculus, excepting the space occupied by special nerve-endings. At the roof of the ampulla, obliquely opposite the apex of the cupula, we find a narrow stripe of cuboid epithelium, which, as a **raphe** (*ra*) extends through the semicircular canals.

Other sections, which did not fall in the axis of the cochlea, will show us the other parts of the membranous labyrinth, sacculus, utriculus and semicircular canals.

Fig. 195.—Ductus Cochlearis of a Young Cat

150. Chromic-osmic-acetic acid. Paraffin section. Iron-alum-hæmatoxylin.

Using a high power lens, we will inspect a single turn of the cochlea. Little can be said about the two scalæ; they are lined by a simple layer of very low, flat cells. The ductus cochlearis does not appear in its natural position in our section, but is turned 90°, so that we must imagine that the cochlear axis is standing erect and to the left side; the scala vestibuli is above, the scala tympani below, the outer wall to the right.

Reissner's Membrane.

Reissner's membrane, separating the ductus cochlearis from the scala vestibuli, arises from the limbus laminæ spiralis (*lsp*), ascends obliquely upward and outward and becomes attached to a small protuberance of the outer wall, the **crista Reissneri** (*cr*). The membrane consists mainly of two layers of cells; the upper one forms the epithelial lining of the scala vestibuli, the lower also consists of flat cells and on both ends of the membrane merges into the epithelium of the outer wall, respectively the tympanic wall. Between the two layers of cells we find scanty connective tissue fibrils.

Ligamentum Spirale.

Of the outer wall of the ductus cochlearis, the *ligamentum spirale*, only part appears in our picture. It forms a crescent-shaped or spherically triangular mass of connective tissue, which is mounted on the inner surface of the bony outer wall. It can be traced above into the *scala vestibuli*, below into the *scala tympani*; three projections are noticed on its inner circumference: the ***crista Reissneri*** (*cr*) at the border between *scala vestibuli* and ductus cochlearis and the ***crista spiralis*** (*csp*) at the border between *scala tympani* and ductus cochlearis. Between these two we can find a rounded protuberance, the ***prominentia spiralis*** (*psp*). It divides the region of the *ligamentum spirale*, situated within the cochlear duct, into an upper part, the ***stria vascularis*** (*strv*), and a lower portion, the ***sulcus spiralis externus*** (*sspe*). The *ligamentum spirale* mainly consists of connective tissue, the bundles of which largely converge toward the *crista spiralis*. The cells, found between the bundles, branch extensively and anastomose among themselves. Between the connective tissue and the epithelium of the ductus cochlearis, in the region of the *stria vascularis* (*strv*) and the *prominentia spiralis*, we have a manifold growth of tissue. Thus we find in the *stria vascularis* a vascular, epithelioid tissue, which is walled off internally by a simple layer of pavement epithelium. At the *prominentia spiralis*, which always carries a small blood-vessel, the connective tissue cells directly gain the surface, pushing aside the epithelial cells. In the *sulcus spiralis externus* (*sspe*) we again find cuboid epithelium, which forms the transitional link to the epithelium of the tympanic wall.

Limbus Laminae Spiralis and the Membrana Tectoria.

We will begin the discussion of the latter from within, starting with the ***limbus laminae spiralis*** (*lsp*). It presents a mass of connective tissue, similar to that of the *ligamentum spirale*, the bundles converging inward, as though they would join those of the *crista spiralis*. The *limbus* projects into the cochlear duct like a mountain, from the summit of which a membrane extends like a bridge over to the epithelium of the tympanic wall; this is the ***membrana tectoria*** (*mt*); the valley, over which it is suspended, is the ***sulcus spiralis internus*** (*sspi*). Below, between the epithelium of the *scala tympani* and the *limbus*, we see the nerve-fibres (*n*) emerging from the *ganglion spirale* and running to the ductus cochlearis. The epithelium of the *limbus* shows different pictures in the various sections. This is due to furrows, found numerously on its surface, which go from within outward and are lined by epithelium. This results in a thick layer, when the section is made deeply in a furrow, a very thin layer, when made superficially. Here, too, we find, as we did in the *prominentia spiralis*, a mixed growth of epithelium and connective tissue. The cuboid epithelium of the *sulcus spiralis internus*, on the one hand, and the ***cells of Claudius*** (*zcl*) joining the epithelium of the *sulcus spiralis externus*, on the other hand, finally bring us to the receiving portion proper of the cochlear epithelium, the ***organ of Corti***.

Organ of Corti.

The *organ of Corti* rests on the **membrana basilaris** (*mb*), stretching from the limbus laminæ spiralis to the crista spiralis. The membrane is a continuation of the connective tissue, constituting these parts; it consists of many radiating, strong fibres, the **auditory strings**, which appear in longitudinal view in our specimen. Sections, which are not made through the cochlear axis, will show them in transverse section. The fibres are connected by a homogeneous mass. Above we have the so-called border-layer, which is especially well reproduced just below the **cells of Claudius** (*zcl*). Below we come to the epithelium of the scala tympani, which is slightly thickened and quite loose, called the **tympanic cover-layer** (*tyb*). Within the latter, in the inner third of the membrana basilaris, we see the cross-section of a vessel, the **vas spirale** (*vsp*).

The elements composing the organ of *Corti* are supported mainly by two rows of cells, the *pillars*, which, closely approximated, ascend in the cochlear duct from the base to the apex of the cochlea. We differentiate between outer (*aepf*) and inner (*ipf*) pillars. Our specimen shows how both arise by broad bases from the membrana propria, soon taper and later again thicken to form a headpiece. While the feet of the pillars are lying at a distance, the headpieces are approximated in a manner that the outer head is placed in a depression of the inner. Between the two pillars a triangular space, **Corti's tunnel**, is left. Its walls have a protoplasmatic lining, which emanates from two cells, situated at the floor of the tunnel, the **floor-cells** (*bz*). High power shows a longitudinal striation in both pillars, due to a deposit of special supporting fibres. The pillars are followed externally by three goblet-shaped cells, placed at short intervals. They do not reach the membrana propria, but end considerably higher. Their upper free end is closed by a coverlike plate, studded with several short hairs. These are the **outer hair-cells** (*achz*) and their *auditory cilia*. The space left between the bases of the hair-cells and the membrana propria is occupied by three cylindrical cells, the **outer stay-cells** (*aestz*); each of the latter sends a phalangeal process between the hair-cells; the processes terminate in head plates, which fit between the plates of the hair-cells. The outer stay-cells, similar to the pillars, also contain supporting fibres. Externally to the last hair-cell the organ of *Corti* declines, forming **Hensen's pad** (*hw*), and finally merges into the cells of *Claudius*. The cells, forming the pad, are long cylindrical and overlap like shingles. The inner pillar is covered internally by a single row of hair-cells. The total of these **inner hair-cells** (*ihz*) is therefore only a third to a fourth of the outer cells. In general, they are like the latter, being surrounded by a dam of **inner stay-cells** (*istz*), which likewise declines internally and merges into the epithelium of the sulcus spiralis internus.

Of the membrana tectoria two portions can be differentiated: a fixed one, imposed on the limbus laminæ spiralis, and a free section, suspended over the sulcus spiralis internus. In the latter portion the membrane attains a considerable thickness, but later becomes thin and closely approximated to the organ of *Corti*; its free, upturned edge comes to an end at the last hair-cell.

A band stretches obliquely across the tunnel of *Corti*; it consists of a

bundle of non-medullated nerve-fibres, which can be traced between the outer pillars to the base of the hair-cells, where the fibres form a plexus, which in turn sends off fibres, terminating at the hair-cells. We can retrace the bundle backward to the trunks, leaving the spiral ganglia. These nerve-trunks lose their medullary sheath before piercing the membrana basilaris by the foramina nervina (*fn*). As stated before, they become continuous with the **tunnel band**, and later break up into bundles, finally reaching the hair-cells.

PLATE 92

Fig. 196.—Olfactory Epithelium of the Sheep

Fig. 197.—Papilla Foliata of the Rabbit

Fig. 196.—Olfactory Epithelium of the Sheep

550. $\frac{3}{4}$. Vital methylene blue process. Chop method.

The olfactory epithelium was presented to us in its gross structure and its relation to the respiratory epithelium during our study of the nares (p. II, 222). We must, however, inquire into some of its details. It can be demonstrated in many ways, the easiest and most efficient being the vital methylene blue staining. The head of one of the large slaughter-animals is used. As soon as possible after death we thoroughly rinse through both carotids (see technique on p. 60, II, 128) all the vessels of the head with warm *Ringer's* solution and then inject 200–500 cm³ of a 1% methylene blue solution at body temperature. An half hour after the injection the head is sawn in two through the median line, and the upper turbinals are fixed in ammonium-molybdate (p. 62). After washing the objects in running water for two hours, we place them successively, during the same day, in 70%, 95% and absolute alcohol. The following day we cut large pieces of the mucous membrane from the bone with the razor, place them again in absolute alcohol for two to three hours and transfer to xylol. We may either embed in paraffin or use the chop method (p. 35). The entire dehydration may be omitted, the chopped specimen being prepared directly from the fixed and washed object. We mount in levulose.

Finer Structure of the Olfactory Epithelium.

The staining is not even throughout. On many places the **olfactory cells** alone have stained blue, on others the **supporting or stay cells** have also taken the color. For our description we select a deeply blue-stained field. The cells are not all evenly stained. Among the great mass of cells we distinguish round or oval cell-bodies, which are mostly seen in the central portion of the nucleated epithelial zone. The fairly large, round or oval nucleus is inclosed by a narrow border of protoplasm, which above and below is continued in a process. The superficial process is the stronger, it curves through the non-nuclear epithelial zone, ending at the surface in a small head, which is studded with several short cilia. The inferior process is thinner; it crosses the nucleated zone of the epithelium and, emerging from the latter, becomes a nerve-fibre of the olfactory nerve. The entire formation constitutes an **olfactory cell** (*rz*); it is a nerve-fibre, transplanted into the epithelium. The **olfactory cilia** (*rh*) extend into or pierce through a homogeneous border, covering the entire surface of the epithelium.

Aside from these olfactory cells we find **stay-cells** (*stz*) in the olfactory mucous membrane. They do not stain as intensely as the former, at times not at all. They are long, cylindrical cells, the nucleus of which is smaller

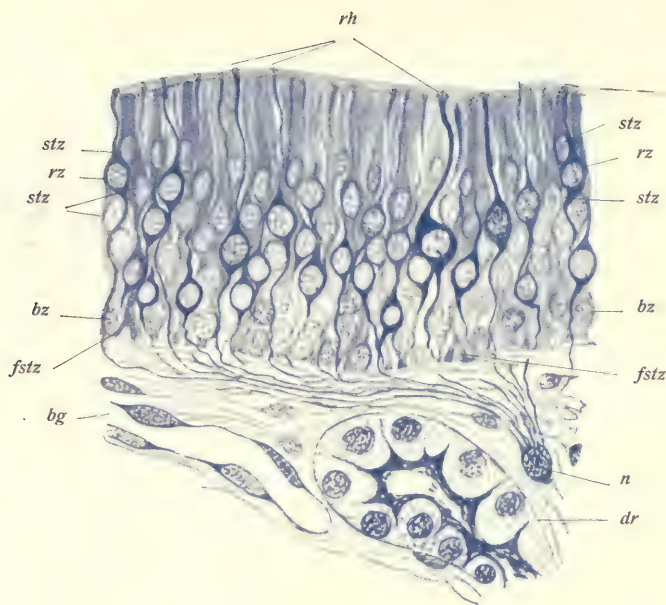


Fig. 196.

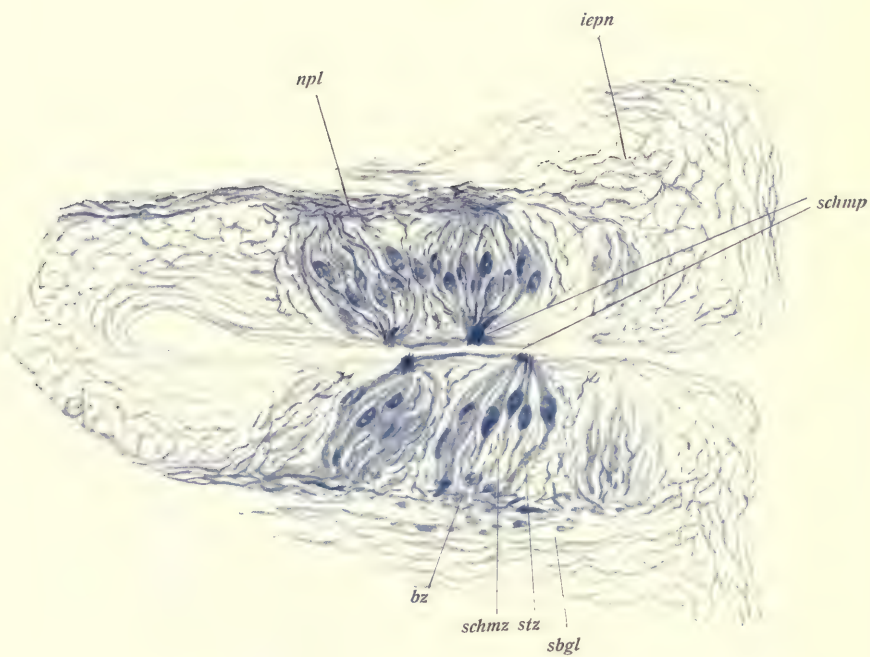


Fig. 197.

than that of the olfactory cells and lies more superficially. The cell-body is narrow, but much broader than the peripheral process of the olfactory cell; beyond the nucleus it becomes narrower and ends in a thickened foot at the beginning of the propria. Very frequently the foot divides (*fstz*). At the epithelial surface a border covers the stay-cells, through which the olfactory cilia penetrate. It appears largely homogeneous, or perhaps shows an indistinct striation, as though it were composed of single cilia or rods.

Finally we find a third variety of cells between the feet of the stay-cells, the so-called **basal-cells** (*bz*), round, cuboid or low cylindrical cells.

The cellular propria harbors many blood-vessels (*bg*) and nerves (*n*). The **glands of Bowman** (*dr*), with their cuboid or conical cells, are also well reproduced. In their lumen we most always find blue dye, excreted by the glandular cells during the injection.

Fig. 197.—Papilla Foliata of the Rabbit

250. Vital methylene blue method. Paraffin section.

The tastebuds, as the typical representatives of the organs of taste, have been presented to us during our consideration of the human tongue (p. II, 181), where we found large numbers of them in the lateral parts of the circumvallate papillæ. To study them more closely, we select the papilla foliata of the rabbit, found at the lateral surface of the posterior portion of the tongue. They assimilate the human very much, but are present in larger numbers.

We employ the vital methylene blue method for their representation, since it not only illustrates the elements, constituting the buds, but also the nerves, entering them. The technique of injection is the same as described on p. 60. Half an hour after completed injection the tongue is excised with the larynx from without. The two papillæ foliatæ are resected with the razor, in a longitudinal direction, viz., bisecting the papillary leaves transversely, and are exposed to the air for one-half to one hour in a moist chamber. Fixation, dehydration and embedding in paraffin, as previously described (p. 62). The paraffin sections are placed parallel to the cut surfaces, so that the papillary leaves will appear in transverse section.

Structure of the Taste Buds.

Not all buds will stain equally well; we must select those that are stained best. Our specimen shows four buds, appearing in their entire length, three others only in partial section. We notice how the buds cross the entire thickness of the epithelial layer, arising by a broad base and ending in a pointed, superficial head, where the bud opens by the **taste-pore** (*schmp*). Below the bud is a lymph-space, the **subgemmal space** (*sbgl*), which also ascends on the sides of the bud.

Similar to what we found in the olfactory mucous membrane, we have again three varieties of cells: the **basal cells** (*bz*), forming the base of the

bud, surmounted by **stay-cells** (*stz*) and **taste-cells**. The former are broader than the latter, lining the interior of the bud, but also found between the taste-cells. Their basal end presents footlike projections, which anastomose with the processes of the basal cells. Their free upper end terminates in a fine point at the floor of the taste-pore. The *taste-cells* are very much like the olfactory cells. Peripherally they terminate in a fine process, the **taste-rod**, which with its fellows forms a bundle of fine threads, projecting from the taste-pore. In one very important respect the taste-cells differ from the olfactory cells. Their basal process is not continued into a nerve-fibre, but ends at the basal cells. Hence we are not dealing with peripheral nerve-cells in this case.

*Nerve-Endings in the
Taste-Buds.*

We can see the nerves ascending everywhere from the submucosa between the leaves of the papillæ and ending in the epithelium (*iepn*). Numerous fibres leave these bundles of nerves and form a dense nerve-plexus (*npl*) beneath each bud. Fibres from the plexus enter the bud itself, ascending within it to the taste-pore with the formation of numerous fine ramifications. Other fibres form a reticulum around the bud, branching between the surrounding epithelial cells.

PLATE 93

**Fig. 198.—Skin from the Palmar Surface of the Human Index
Finger**

**Fig. 199.—Skin from the Palmar Surface of the Human Index
Finger**

9. THE SKIN.

Fig. 198.—Skin from the Palmar Surface of the Human Index Finger

35. $\frac{3}{4}$. Sublimate. Frozen section. Iron-hæmatoxylin. Resorcin fuchsin. Picrofuchsin.

Since the various layers of the skin differ in regard to development in different parts of the body-surface, it is advisable to compare sections from several regions. We will first describe a section through the palmar surface of the finger, which contains the various layers of the skin in a state of high development. Pieces, about 5 mm in thickness, are cut from the palmar surface of the distal phalanx, reaching down to the bone; they are fixed five to six hours in a 3% solution of sublimate, washed overnight in water and placed in 5% formalin. Formalin fixation alone also gives good results. Thin frozen sections are first stained in iron-hæmatoxylin (p. 57) for five to ten minutes, washed in water, transferred to 70% alcohol and counterstained in resorcin fuchsin for fifteen to twenty minutes (p. 63). After thoroughly washing in 95% alcohol, we place the sections for ten minutes in a vessel, containing picrofuchsin (p. 67); they are then rinsed in 70% alcohol, dehydrated, and after xylol are mounted in balsam.

Such a section shows primarily that the outer skin, similar to mucous membranes within the body, consists of an external epithelial portion, the **epidermis**, and an internal connective tissue part, the **cutis**. The boundary between these two is not a simple, smooth line, the cutis forming macroscopic **shelves**, easily recognized in our specimen, which are separated by shallow depressions and studded with two rows of tuftlike projections, the **papillae**. The epidermis covers the cutis-shelves in such a manner that the interposed recesses can be noticed externally (*l* and *s*). The papillæ, on the other hand, do not appear externally, since the epidermis not only dips in between them, but also covers them in a huge cellular layer.

Epidermis.

The epidermis, a high layer of stratified flat epithelium, composed of numerous strata of cells, shows four zones, differing distinctly in color. Outermost we have the thickest layer, the bright yellow **stratum corneum** (*strco*), followed internally by a much narrower, yellowish-brown stripe, the **stratum lucidum** (*strlu*). These two layers, composing by far the greatest part of the entire epidermis, are distinguished by the fact that no nuclei can be seen. Neither can we find any nuclei in the next layer with our method of staining. This **stratum granulosum** (*strgr*) is very narrow in this case; its cells, arranged in one or two layers, have taken a black color,

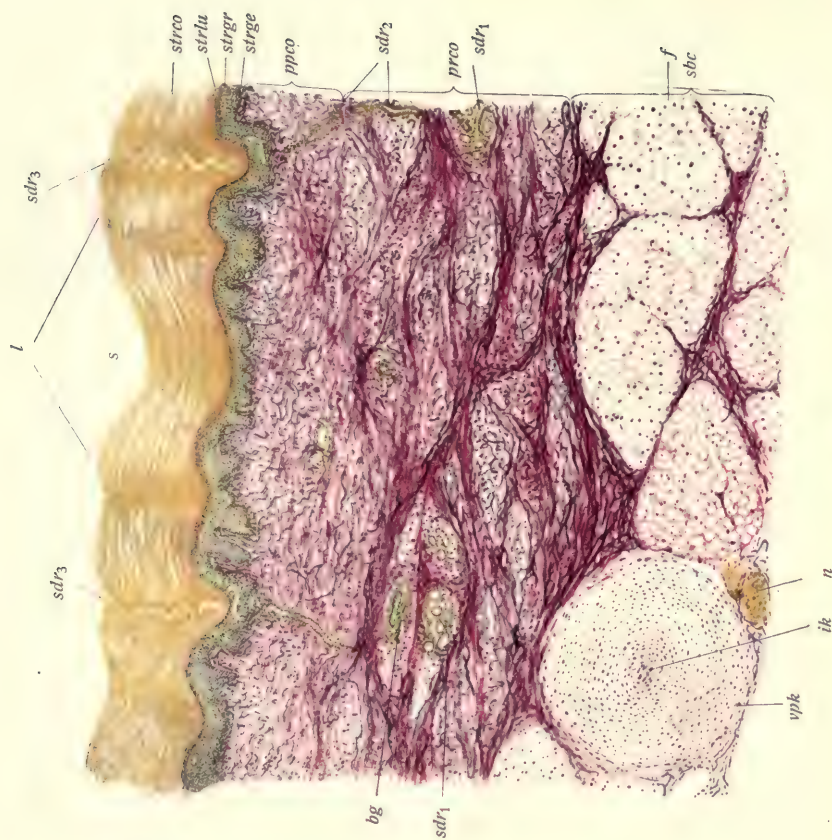


Fig. 198.

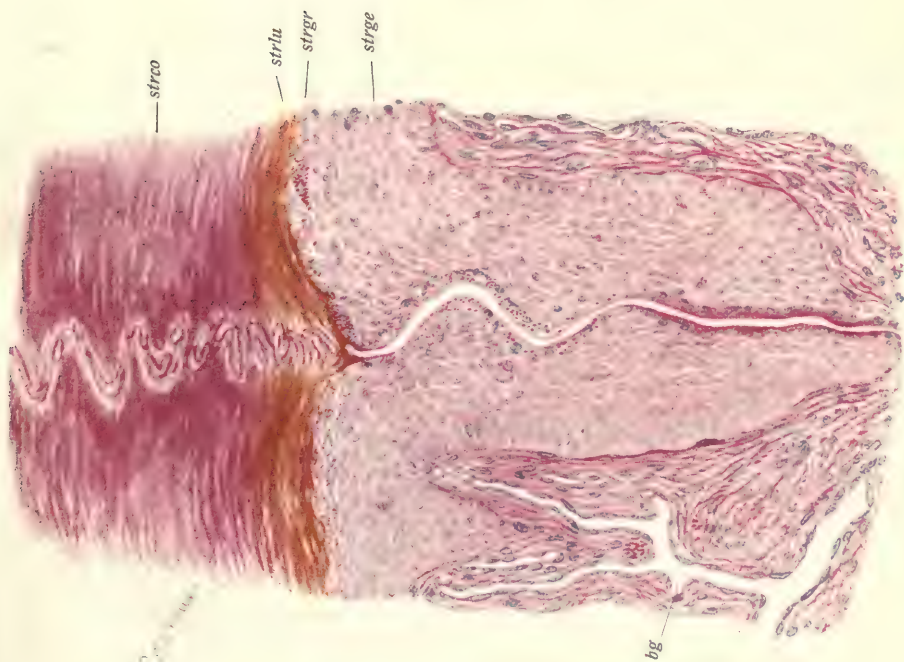


Fig. 199.

forming a black, undulating line, bounding the next layer, the **stratum germinativum** (*strge*) externally. The germinal layer fills out the valleys between the papillæ, showing distinct, nucleated cells.

Cutis.

The cutis, the connective tissue portion of the skin, is divided into a superficial part, the **corium**, and the deeper subcutaneous connective tissue. The arrangement of the connective tissue bundles of the corium separates it into two different zones. Beneath the epidermis, in the shelves and papillæ, the tissues are very dense; the connective tissue bundles, crossing in all directions, leave only minute interspaces. This outer part of the corium has been called the **pars papillaris** (*ppco*). The picture changes as we progress more deeply. The interlacing bundles leave larger interspaces, called **Langer's rhombs**. To this deeper portion of the corium the name **pars reticularis**, or often the misnomer, *tunica propria*, has been given. The corium gradually becomes very loose, the rhombs growing larger and the connective tissue bundles smaller, and finally merges into the **subcutaneous connective tissue** (*sbc*), which connects the skin with the underlying organs. The connective tissue of the skin is extensively blended with elastic fibres, which often form a network. We notice that these fibres are thicker below, gradually tapering toward the surface. In general they take the same course as the connective tissue bundles; in the *pars papillaris* they ascend vertically toward the epidermis, where they form the delicately constructed, subepithelial net of elastic fibres.

The rhombs of the *pars reticularis* of the corium are filled either with fat or glandular tissue, the bodies of the **sudoriferous glands** (*sdr₁*). One duct (*sdr₂*) leaves each collection of glands, traversing the *pars papillaris* vertically or obliquely, and enters the epidermis always at the floor of an epidermal peg. In the epidermis it takes the characteristic spiral, corkscrew-shaped course (*sdr₃*), opening at the summit of the cutaneous shelves.

In the subcutaneous tissue we find more or less fat (*f*), depending upon the state of nutrition of the individual. Here, too, we find **Pater-Pacinian corpuscles**, touch-corpuscles. The nerve, going through the axis of the corpuscle, is surrounded, as by a cloak, by several layers of flat cells. This **inner piston** (*ik*) is in turn surrounded by numerous (up to 60) connective tissue membranes, lined by simple, flat epithelium, the succeeding one always enclosing the preceding one completely. Aside from the transverse section of the corpuscle our figure also shows two small nerve-trunks (*n*).

Fig. 199.—Skin from the Palmar Surface of the Human Index Finger

300. $\frac{3}{4}$. Sublimate. Frozen section. *Biondi* solution.

Sections are stained with *Biondi* solution (p. 67) for examination with high power.

*Detailed Structure
of Epidermis.*

This stain brings out the various layers of the epidermis. The stratum corneum (*strco*) stains intensely red. It consists of thin, scaly cells; nuclei cannot be found. A high power immersion lens will show that in each cell a deeper red outer zone surrounds a pale red inner layer. The outer layer consists of **horny substance**, keratine; the hornlike, solid covering encloses liquid contents. In the stratum lucidum we can barely recognize some cells, this layer staining more orange in contradistinction to the stratum corneum. It contains a substance, which is distinguished by a strong lustre and double-refraction, the **eleidin**, which, even genetically, has probably nothing to do with keratine. It is a product of **keratohyalin**, which fills the cells of the stratum granulosum in form of fine granules. These cells are elongated, spindle-shaped cells, arranged in two or three layers. Each cell contains a well-developed nucleus. The granules are small and round, filling the cell-body entirely. If the specimen be stained with basic dyes exclusively, the granules will eagerly take up the stain. They have therefore been called basophilic. This basophilia may be compared with that of *Nissl's* granules (p. II, 121); it is only apparent; stained with heterogeneous mixtures, the granules will always select the acid dye. The stratum germinativum (*strge*) consists of a deeply situated, simple layer of cuboid or cylindrical cells; hence it has also been described as a separate, deepest layer of the epidermis, the **stratum cylindricum**. The membrana propria is wanting, the connective tissue fibres of the corium piercing more or less between the cylindrical cells of the epidermis. The entire remaining bulk of cells of the stratum germinativum is also termed **stratum spinosum**, because these cells are typical bristle-cells, i.e., protoplasmatic fibres run through the outer stratum of cells, which are separated by intercellular spaces, such as we had occasion to observe previously (p. II, 46).

Ducts of the Sweat-Glands

A sudoriferous duct is seen in our specimen, piercing the epidermis. Its wall in the outer parts of the stratum germinativum is formed by the cells of the stratum granulosum, which are arranged along the duct, appearing homogeneous in that portion, which borders directly on the lumen. More deeply in the epidermis the cells of the stratum spinosum itself form the lining. They also appear homogeneous and are strikingly different in appearance from the surrounding cells. Within the horny layer the duct lacks a lining, but appears to have a structureless coat, which generally retracts from the adjacent mass of horny cells during the preparation of the specimen.

In the corium papilla, shown alongside, a blood-vessel is seen (*bg*), which terminates in loop-shaped capillaries.

PLATE 94

Fig. 200.—Sudoriferous Glands from the Human Skin

Fig. 201.—Transverse Section through the Human Scalp

Fig. 200.—Sudoriferous Glands from the Human Skin

450. $\frac{3}{4}$. Sublimite. Frozen section. *Biondi* solution.

Finer Structures of the Sudoriferous Glands.

Selecting a deep portion of the skin, we will study the structure of the sudoriferous gland under high power. We find the various turns of the canal partly in longitudinal, partly in transverse section. The lumen, much larger here than in the efferent duct, is lined by high cuboid cells, the bodies of which show an irregular meshwork of protoplasm. At their free edge the cells are covered by an intensely red, homogeneous cuticular border, which also dips in between the heads of the cells in form of pegs. Numerous **secretory capillaries** extend from the lumen deeply down between the cells.

Externally the secretory epithelium is covered by a layer of flat nuclei, which, as we may easily recognize in cross-sections, belong to **smooth muscle cells**, enclosing the wall of the tubule in oblique spiral turns. The form of these contractile elements is nicely illustrated at (*m*). The tubular wall has only been partially cut, and the outlines of the epithelial cells are seen shining through, being covered by a layer of spindle-shaped, branching muscle-cells. The latter again are externally coated by a thin, homogeneous **membrana propria**.

The picture takes on essential changes, where the secreting tubule merges into the excretory duct (\times). The muscle-cells disappear, the *membrana propria* remaining as an outer covering. The epithelial cells gradually become lower, a second layer of flat cells appearing above them, which stain deeper red and at the lumen merge into a deep red cuticular border. At (*ag*) the same duct appears in transverse section, illustrating the double layer of epithelium and the characteristic triangular lumen. Between the different sections of the glandular canal we find numerous blood-vessels; mast-cells (*maz*) are also present.

Fig. 201.—Transverse Section through the Human Scalp

20. $\frac{3}{4}$. Formalin. Frozen section. Safranin. Picroindigcarmin.

For the demonstration of the structure and arrangement of the hairs, larger pieces of the human scalp are used. The hair is cut short and the specimen mounted on a wax plate, hair down; fixation in 10% formalin for two days and equally long in 5% formalin. Controlling through a magnifying lens, we cut narrow strips in such a manner that the intracutaneous portion of the hair is cut exactly longitudinally, as far as this is possible.



Fig. 200.

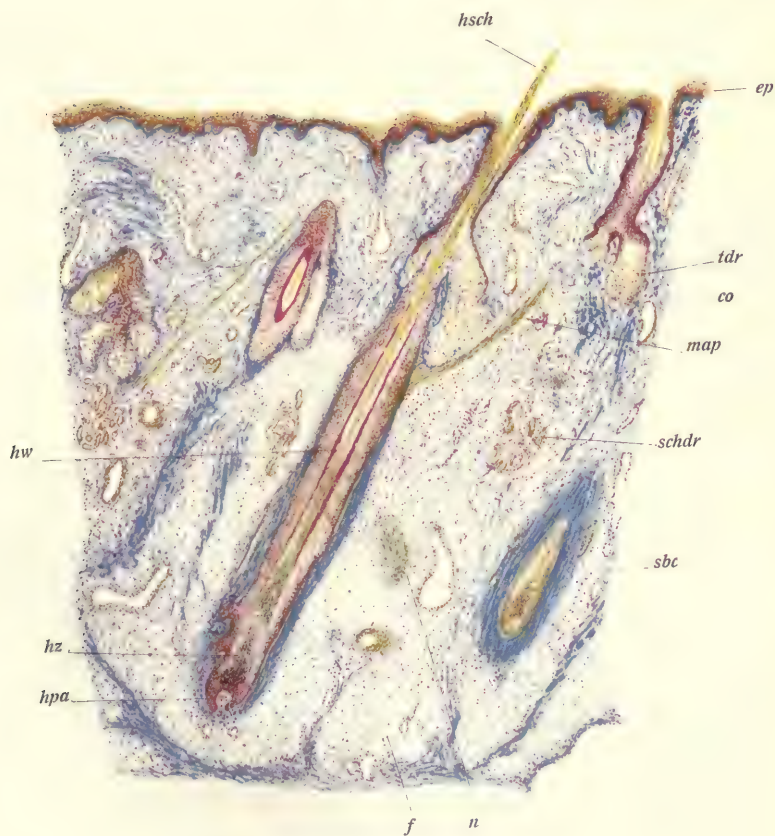


Fig. 201.

Frozen sections are made in the same direction and stained in safranin (p. 63) for one to two hours; wash well in 95% alcohol and counterstain in picroindigocarmin (p. 67) five to ten minutes. After thoroughly washing in 70% alcohol the sections are completely dehydrated in absolute alcohol and through xylol mounted in Canada balsam.

*General View of the
Structure of Hair.*

Our specimen shows a hair in its entire length. It is embedded in the skin in an oblique, not a vertical direction. It presents a **shaft**, projecting from the scalp (*hsch*), a **root** (*hw*), embedded in the **corium** (*co*), and a club-shaped ending, the **bulb** (*hz*), extending into the subcutaneous tissue (*sbc*). The hair has invaginated the surface epithelium, the epidermis (*ep*), the latter surrounding the hair with special cellular sheaths, the **hair-root sheaths**. Our specimen clearly shows a narrow, deep red, **inner root-sheath** and a broad, yellowish-red **outer root-sheath**. At the hair-bulb the elements of the hair become continuous with those of the root-sheaths. Vascular connective tissue pierces into the hair-bulbs from below, forming a button-shaped invagination, the **hair-papilla** (*hpa*). The root-sheaths surrounding the hair are separated from the corium by a strong connective tissue coat, the **hair follicle**.

Sebaceous Glands.

From the upper portion of the hair follicle large, racemose glandular masses pierce into the corium, the sebaceous glands (*tdr*), each hair having at least one. Their cellular coat is a continuation of the outer root-sheath. The cells have increased in number, filling almost the entire lumen of the gland. Fine fat-globules are elaborated within the cells, hence these glands will always appear bright red when stained with sudan (compare, e.g., Fig. 193). When the fat-globules have been extracted by our method of treatment (alcohol, xylol), a distinctly net-form protoplasm remains in the cells, such as we have seen in our specimen under high power. As we leave the periphery of the gland and gain the interior, the cells become more fatty, the nuclei more rudimentary. In the centre of the gland the cells disintegrate, the débris and the now free fat-globules forming the glandular secretion, the **sebum**.

Below the sebaceous gland of the longitudinally cut hair we notice a yellowish-green band (*map*), descending from the superficial portion of the corium in a course, which is slightly more oblique than that of the hair, and becoming inserted in the hair-follicle; in so doing, it surrounds the end of the sebaceous gland. This is a bundle of smooth muscle fibres, the **arrector pili**. It comes from the stratum papillare of the corium; when contracting it will cause the hair to become more erect and to protrude farther from the scalp (gooseskin); furthermore, it will press on the sebaceous gland and cause the secretion to be poured out.

Aside from a number of blood-vessels and nerves (*n*), our specimen shows an abundance of fat (*f*) and numerous sudoriferous glands (*schdr*).

PLATE 95

Fig. 202.—Longitudinal Section through the Human Hair-Root

Fig. 203.—Flat Section through the Human Scalp

Fig. 202.—Longitudinal Section through the Human Hair-Root

150. $\frac{3}{4}$. Formalin. Frozen section. Safranin. Picroindigocarmin.

Detail Structure of the Hair.

We shall now make a close study of the lower portion of the hair, hair-root and hair-bulb, with a medium strength objective. First we come to the connective tissue **hair-follicle**. It consists of connective tissue fibres, arranged in bundles, which externally run in longitudinal, internally in circular direction. It contains numerous blood-vessels and nerves; the former never transgress the region of the follicle internally, the latter may advance to the outer root-sheath. Internally the follicle is bounded by the **glass membrane** (*glhb*), which, in our specimen, is clearly defined by its green color from the blue connective tissue of the hair-follicle. While the external outline is smooth, it sends numerous processes inward between the cells of the external root-sheath.

The **outer root-sheath** consists of numerous cellular layers, attaining the greatest thickness at the centre of the hair-root, tapering above and below. Quite often the outline is not smooth, but presents budlike protuberances, projecting into the hair-follicle. Similar to the germinal layer of the epidermis, we may differentiate between an outer stratum cylindricum and an inner stratum spinosum.

The **inner root-sheath** is much more complicated in structure. Primarily it is made up of two different layers, which cannot be equally well differentiated throughout. They are well demonstrated in the deeper portions of the hair-root, just above the bulb. The outer, **Henle's layer** (*hesch*), appears as a simple layer of narrow cells, containing narrow, flattened nuclei. Below, the cells become lost in the hair-bulb; above, the formerly green cells suddenly stained red with safranin, viz., they are keratosed. **Huxley's layer** (*hæsch*) of the inner root-sheath is materially broader than *Henle's*. It consists of two to three layers of cuboid cells; the nuclei, in the lower portion of the root, are rounded or oval; farther above they undergo various changes in shape. The cell-body encloses bright green globules of **kerato-hyaline**, such as we met in the cells of the stratum granulosum of the epidermis. The globules become smaller below, more distinct and stain brighter, while above they grow larger, blurred, fainter in color; they become confluent and finally disappear altogether, as soon as keratosis sets in in the cells. The cells of *Henle's* layer also contain keratohyaline, but do not present such distinct globules; the narrow cells stain green *in toto*. Keratosis manifests itself earlier in *Henle's* layer than in *Huxley's*, thus the latter appears for a distance surrounded by a red band, composed of the former. After *Huxley's* layer has likewise undergone horny degeneration, both strata

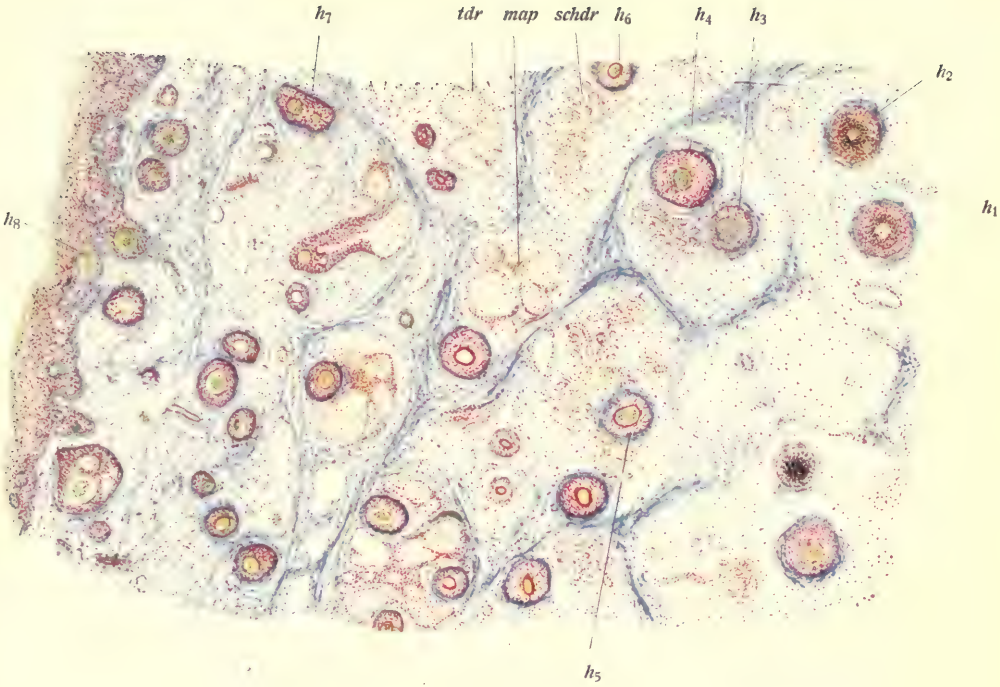


Fig. 203.

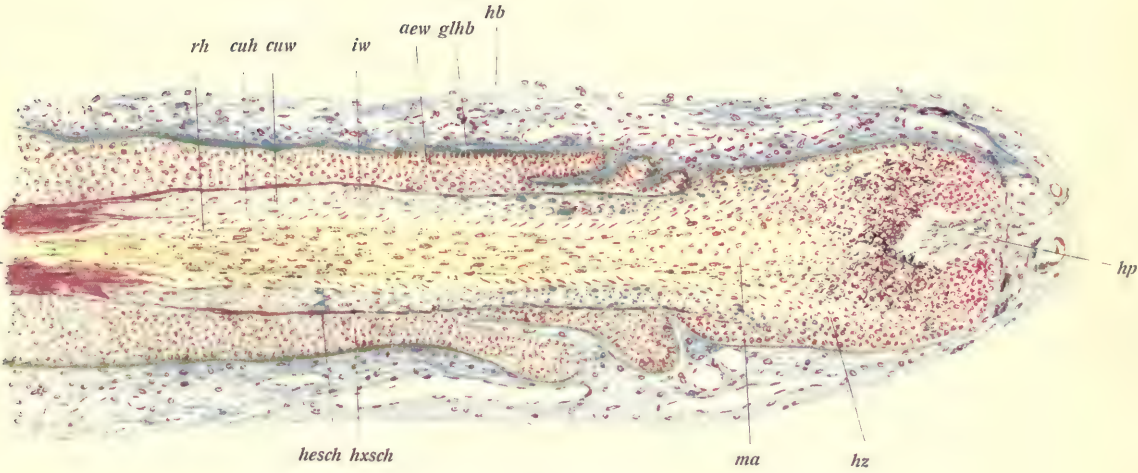


Fig. 202.

join to form a strong red band, which we so distinctly noticed in the preceding specimen at the root. The band becomes lost near the mouth of the sebaceous gland. Internally the root-sheaths are bounded by the **cuticula of the root-sheaths** (*curv*), a simple layer of flat cells. —

The *hair* itself arises from the **hair-bulb** (*hz*), where all layers of the hair as well as the root-sheaths come together. The bulb consists of a powerful, stratified cellular mass, which is enveloped by the vascular connective tissue papilla. In dark individuals the cells are always pigmented. From the cellular layer, surrounding the papilla, develops the **medullary substance** of the hair (*ma*), a string of cuboid cells, arranged in the axis of the hair. It is not very well demonstrated in our specimen, due to the fact that the section does not fall exactly in the axis of the hair. Externally the bulk of the hair is formed by the **cortical substance** (*rh*). It consists of long, spindle-shaped, nucleated cells, which can be demonstrated by heating an eyelash in a drop of concentrated sulphuric acid on a slide, covering it with a cover-glass and isolating the cells by rocking. As shown in our specimen, these cells of the cortex contain pigment, which determines the color of the hair. It is deposited in the cells in form of fine globules, being continued also into the cells of the bulb. In the hair-papilla we likewise find pigment, enclosed in branching fibroblasts. Externally the hair is bounded by the **cuticula of the hair** (*cuh*). It is composed of nucleated, rectangular, scale-shaped cells, which are arranged like shingles of a roof; since the free, upper border of the cell is not straight, but rounded, undulating lines, approximately parallel to one another, are seen through the microscope, running at right angles to the long axis.

Fig. 203.—Flat Section through the Human Scalp

35. $\frac{3}{4}$. Formalin. Frozen section. Safranin. Picroindigcarmin.

In order to obtain good transverse sections through the hair, we must not simply cut parallel to the skin surface; that method would result in oblique sections. The best plan is to excise square pieces from the scalp, fixed in formalin; using a magnifying lens, we determine the course of the hair at the lateral surfaces; with the razor we now remove so much of the under surface of the cutis of the scalp on one side that the hairs of the piece of scalp, now lying on the freezing-table, are vertical. The sections are treated in the same manner as in the preceding specimen.

Arrangement of the Hairs in the Scalp.

Such an oblique flat section through the skin must necessarily furnish transverse sections through the hairs at the different heights of their implantation in the scalp. Our specimen shows how in the scalp the hairs are always grouped in numbers, two to five together, surrounded by large masses of fat; each group of hairs is separated from the next by strong connective tissue septa, arising from the cutis of the scalp. In the subcutaneous con-

nective tissue we find, aside from the transverse sections through the hair-root, a collection of sudoriferous glands (*schdr*), which in the lower portions of the corium are replaced by sebaceous glands (*tdr*), which disappear in the upper parts of the corium. We have numbered the various cross-sections of hairs in our picture according to their height. At h_1 the section has crossed the hair-bulb, so that the blue-stained connective tissue of the papilla appears in the centre; h_2 shows a place just above the papilla; h_3 goes through the root of the hair, the green ring representing the two layers of the inner root-sheath, filled with granules of keratohyaline. At h_4 and especially at h_5 we have reached the keratosed part of *Henle's* and part of *Huxley's* layers, and h_6 shows the fully keratosed inner root-sheath; h_7 demonstrates two thin hairs in a common follicle, and h_8 finally brings before us the hair-shaft within the epidermis. The arrector pili muscles (*map*) are illustrated in several places.

PLATE 96

**Fig. 204.—Transverse Section through the Distal Phalanx of a
Child's Finger**

Fig. 204.—Transverse Section through the Distal Phalanx of a Child's Finger

18. Formalin. Frozen section. *Biondi* solution.

For the study of the structure of the nail, sections through the distal phalanges of the fingers of newborn infants, or such of a few days of age, are best suited. The nail of adults is hard to cut. The phalanx is fixed in 10% formalin, transferred after twenty-four hours to 5% formalin, and sectioned on the freezing microtome into transverse and longitudinal sections. The cutting does not offer any difficulties, since the bone contains but little lime. The sections are stained in *Biondi* solution (p. 67). Transverse sections are generally more demonstrative than longitudinal.

Our specimen shows the transversely cut bony ring of the distal phalanx (*kn*) with its narrow cavity (*mah*), which is filled with bone marrow. The ring of bone is surrounded by a connective tissue periosteum, very rich in cells at its deeper layers, which externally merges into the subcutaneous connective tissue of the finger. In the region of the palmar surface numerous connective tissue septa radiate outwardly to the corium. In the compartments of the finger-tip, thus formed, we find numerous blood-vessels as well as strong nerve-trunks, together with *Pater-Pacini tactile corpuscles* (*vpk*). They occupy the deeper portions of the subcutaneous tissue, being followed externally by masses of *sudoriferous glands* (*schdr*), the long tortuous ducts of which we may observe throughout the subcutaneous tissue, the corium and the papillæ of the epidermis.

Structure of the Nail.

The skin is elevated on either side, forming the *nail-fold* (*naw*). The slope on either side of the median line brings us to the *nail-furrow* (*naf*). The entire centre is occupied by the *nail-bed* (*nab*). A slight mass of connective tissue, intimately connected with the periosteum of the terminal phalanx, arises dorsally in regular, high, longitudinal bands, which in our transverse section appear as comb-shaped formations. At the region of the root of the nail these bands or tracts are lower, becoming higher anteriorly. The interspaces between the tracts are occupied by the *germinal layer* of the nail (*k*); the latter also covers the surface of the tracts in a thin continuous layer, merging into the stratum germinativum of the skin at the nail-furrow. As the tracts become flatter posteriorly, the germinal layer increases in size and at the root appears covered by a stratum granulosum. The latter prevents the capillaries of the nail-bed from being seen, so that the nail in this region appears whitish externally (*lunula*).

The *body of the nail* (*nak*) rests on the germinal layer; this is the



Fig. 204.

nail proper. Posteriorly it becomes thinner and merges into the **nail-root**, anteriorly it ends in a free edge, laterally the sharpened edge of the matrix ends in the furrows. In the latter the horny layer of the epidermis is reflected over the nail, forming a thin membrane, the **eponychium** (*epo*), which covers the nail in part. The under surface of the nail is invested with a similar **hyponychium**. The nail proper consists of flat, scalelike, horny cells, which form horny leaves, arranged in shingle-fashion. These horny scales can readily be isolated by macerating the nail for several hours in a solution of potassium hydroxide (p. 35).

PLATE 97

Fig. 205.—Flat Section through the Waxskin of the Duck's Beak

**Fig. 206.—Transverse Section through the Waxskin of the
Duck's Beak**

Fig. 205.—Flat Section through the Waxskin of the Duck's Beak

35. $\frac{3}{4}$. Vital methylene blue method.

The sensory nerve-endings in the skin are very manifold, at one time the nerves ending freely between the cells of the epidermis, as we have seen in the cornea (p. II, 358), at others we have the formation of special end-corpuscles, which may be situated in the stratum papillare, the corium or the subcutaneous connective tissue. Such corpuscles we have already met in the human skin as *Pater-Pacinian* corpuscles (pp. II, 379, 390).

A very excellent object for the study of the nerve-corpuscles is the beak of the duck. Here we find such corpuscles in large numbers in the waxskin and the mucous membrane of the gums. The respective nerves can be demonstrated by the vital methylene blue staining process in the following manner. A duck is killed with chloroform, the thorax opened by dividing the ribs on either side with bone-scissors and everting the manubrium, including the large breast-muscles, upward. After opening the heart by cutting off the apex and removing the protruding blood by sponging, a wide cannula, filled with warm *Ringer's* fluid, is tied in the left ventricle. The rinsing and injection can now be commenced according to the previously stated rules (p. 60); however, it is a good precaution to first tie the descending aorta within the thorax as well as the subclavian artery of either side below the origin of the very thin common carotid. In that way most of the dyestuff is directed toward the head, and the result of the staining will be better.

Half an hour after completion of the injection the upper part of the beak is removed. The waxskin is taken off from the outer edge of the beak down to the bone and pieces of it are placed in the moist chamber. Very suitable are also the edges of the upper and lower beak with their well-known horny lamellæ. The horny lamellæ are first removed with the razor and then the entire plate is taken off. The gums, cut away with the scalpel all around, should also be used. When removing the latter we expose the large branches of the trigeminus supplying the beak. After assuring ourselves under the microscope of the thorough bluing of the nerves, the specimens are fixed in the usual manner (p. 62). Waxskin and gums are dehydrated, embedded in paraffin, and sectioned. From the lamellar plate we make flat sections, after dehydration and the use of xylol, which are mounted in Canada balsam.

Distribution of the Nerves in the Duck's Beak.

Our figure represents such a surface specimen; in the superficial parts we may still recognize the remains of the yellowish horny lamellæ. The thick nerve-trunks, entering the skin, break up into numerous branches, which divide in turn, thus forming an extremely dense plexus of nerves. Fine fibrils



Fig. 205.



Fig. 206.

arise from it, which either end freely in the epithelium or enter nerve-end corpuscles, of which we are able to distinguish two varieties here. The nerve-fibre may either end in the axis of a large lamellar body, the **Herbstian corpuscle** (*hk*), or it terminates in the form of a small, rounded plate, **Grandry's corpuscle** (*gk*).

These specimens also demonstrate the blood-vessels (*bg*) very well in some places. The smooth muscle of the latter stains well; also the nerves supplying them can often be traced to their endings.

Fig. 206.—Transverse Section through the Waxskin of the Duck's Beak

190. $\frac{3}{4}$. Vital methylene blue stain. Paraffin section. Paracarmin.

The structural details of these corpuscles we will study on a section through the waxskin. The sections are made vertically to the surface, 15–20 μ thick. Without being pasted on first, the paraffin sections are brought from the knife directly to a dish of xylol, thereafter to absolute and 90% alcohol. They are now stained for one to two minutes in paracarmin (p. 55), washed in absolute alcohol, and mounted through xylol in Canada balsam.

Herbstian Corpuscles.

Our specimen shows two *Herbstian* corpuscles, one in longitudinal section (*hk*₁), the other in transverse section (*hk*₂). Similar to our findings in the *Pater-Pacinian* corpuscles, we again notice numerous connective tissue lamellæ, one invaginated in another, and covered on their inner surface by epithelioid cells. The lamellæ, which are by no means as numerous as in the *Pater-Pacinian* corpuscles, are found at great intervals externally, but further inward become more closely aggregated, thus imparting to the inner portion of the capsule a more homogeneous appearance, which is increased by the disappearance of the lining cells.

A **medullated nerve-fibre** enters the corpuscle, pierces the capsular membranes and, losing its medullary sheath, arrives at the axis of the corpuscle. It runs along the latter and ends within it in a pyriform swelling. At both sides of the **axis-cylinder** we have a row of cells, which perform the rôle of **touch-cells**, forming in their entirety the so-called **inner piston**. In well-stained specimens we may recognize short side-branches, leaving the axis-cylinder and ending between the cells of the inner piston. Thus we have here a very similar structure to that of the *Pater-Pacinian* corpuscles, the cells in the inner piston of the latter being flatter and less regularly arranged.

Grandry's Corpuscles.

These (*gk*) are entirely different in construction. They only possess two, but very large touch-cells of biscuit shape, the flat surfaces of which are closely approximated. The lamellar boxes have disappeared, a thin con-

nective tissue capsule surrounding the corpuscle. Here, too, a medullated nerve-fibre enters the corpuscle, losing its myelin sheath when piercing the capsule. The denuded axis-cylinder broadens out in the form of a round, flat disk, the **touch-disk**, situated between the two touch-cells. The disk lies parallel to the surface of the waxskin, thus appearing in our cross-section like a narrow, blue stripe. In the preceding flat section we were able to see it in its true shape; there we can recognize, with high power, that we are not dealing with a homogeneous disk, but with the axis-cylinder, which has spread to form a large network of fibrils. *Grandry's* corpuscles may vary, inasmuch as three, four or five cells instead of two can constitute a corpuscle. Such a corpuscle, called a **columnar corpuscle**, will accordingly have two, three or four end-plates, which, however, are supplied by the same nerve-fibre.

Our specimen shows how the nerve-fibres, leaving the deeply situated trunks of the nerve-plexus (*n*), enter the corpuscle. They are accompanied by fibres, which, piercing the corium papillæ, enter the epidermis and end freely between the cells (*epm*).

PLATE 98

Fig. 207.—Functionating Human Mammary Gland

Fig. 208.—Functionating Human Mammary Gland

Fig. 207.—Functionating Human Mammary Gland

35. Formalin. Frozen section. Iron-hæmatoxylin. Sudan. Picrofuchsin.

To study the mammary gland during function, we select the glands of women who have died during lactation. Moderately sized pieces of the organ are fixed in 10% formalin for twenty-four hours, transferred to 5% formalin, and cut on the freezing microtome. The sections are first stained in iron-hæmatoxylin (p. 57) for five minutes, rinsed in hydrant water, transferred to 50% alcohol, and then counterstained in an alcoholic solution of sudan (p. 66). After fifteen minutes the sections are rinsed in water and again counterstained in picrofuchsin (p. 67). The excess dye is rinsed off in water and the sections mounted in levulose.

Structure of the Functionating Mammary Gland.

Each large mammary lobe is composed of numerous small lobules, which are separated by strong connective tissue septa. Our specimen presents eight lobules (*drl*). The surrounding fatty (*f*) connective tissue contains many blood-vessels (*bg*) and the **interlobular ducts** (*afg₁*). The latter receive branches from the lobules. One of these (*afg₂*) has been cut longitudinally, showing the branches, given off, numerous tortuous canaliculi, which at their termination enlarge to a sort of alveolus.

The epithelium, lining the ducts, is simple cuboid. As the ducts grow larger in calibre, the cells become more cylindrical. By the confluence of the tubules of each lobe a **milk-duct** is formed, in all about twenty being present in one gland; they open at the nipple, having previously enlarged in a **milk-saccule**. In the latter the cylindrical epithelium is replaced by the stratified epithelium of the nipple.

Fig. 208.—Functionating Human Mammary Gland

600. Formalin. Frozen section. Sudan. *Biondi* solution.

To acquaint ourselves with the finer structure of the secreting end-pieces, we place a thin frozen section in 50% alcohol, stain for fifteen minutes in an alcoholic sudan-solution (p. 66), wash in water, and counterstain in *Biondi* solution (p. 67). Mounting in levulose.

The Secreting Epithelium of the Mammary Gland.

Our picture shows such a glandular end-piece. It is lined by a simple layer of cuboid cells, which contain numerous **fat-globules**, stained yel-

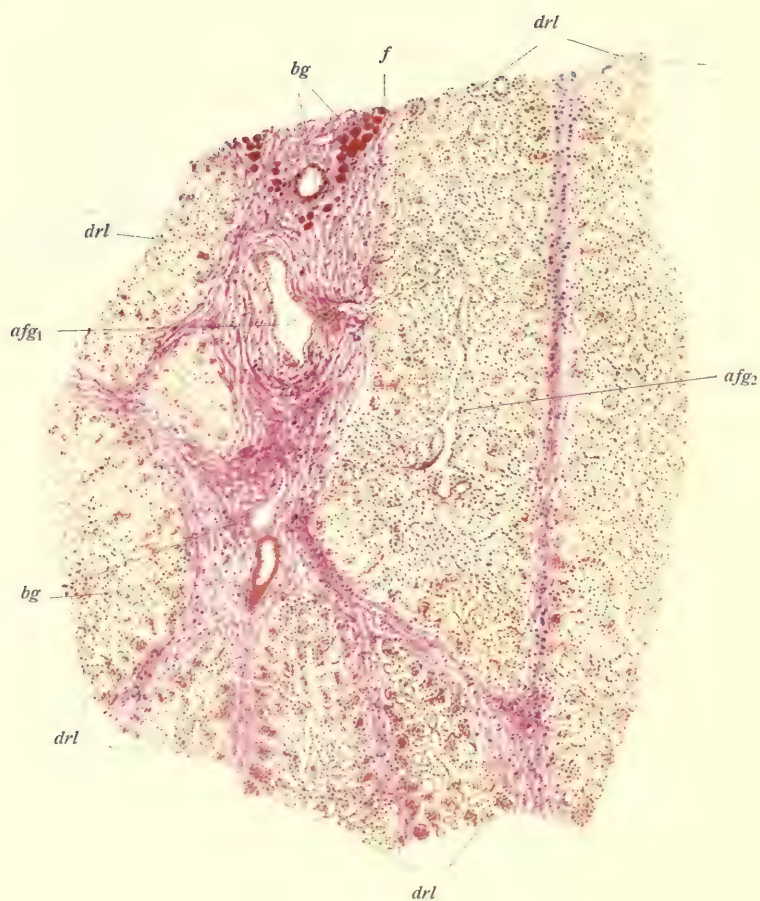


Fig. 207.

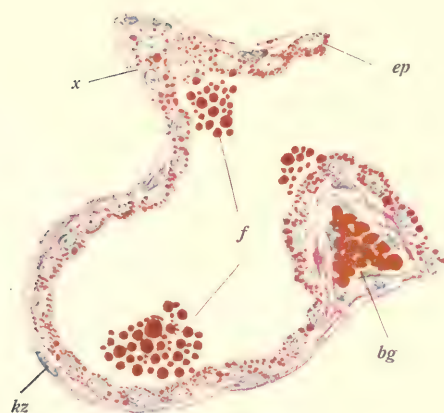


Fig. 208.

lowish red by the sudan. The nuclei appear oval in cross-section, round in the flat section (X). Fat globules are present in all sizes, from the size of dust grain to voluminous drops. The latter are formed by gradual confluence of the former and may be present in such quantities as to push the inner portion of the cell in the glandular lumen in club-shape. They afterward burst into the lumen (f), forming an important part of the *milk*.

Externally the epithelium of the alveoli is covered by branching cells (kz), the processes of which anastomose and form a protoplasmatic network, which covers the surface of the alveoli. We thus have here *basket-cells*, so-called, such as we met in the salivary glands. The basket-cells are covered by a structureless *membrana propria*. Both basket-cells and *membrana propria* are continued in the ducts. In the connective tissue, surrounding the alveoli, we find many migrating cells.

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